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YIELD, COMPOSITION, AND OTHER LATEX CHARACTERISTICS OF CRYPTOSTEGIA GRANDIFLORA[1]

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INTRODUCTION

Cryptostegia grandiflora R. Br., a member of the Asclepiadaceae, has long been known as a producer of rubber. This climbing shrub, which is tolerant of a wide diversity of soils and climates, grows as an escape in tropical and subtropical parts of Mexico and Central America as well as under cultivation in southern Florida, Texas, and California. It was a sufficiently promising war-emergency source of rubber to encourage further investigation. This paper presents results of some of the studies made by the United States Cryptostegia Research Laboratory, Ciudad Victoria, Tamaulipas, Mexico, during 1942–45.[3]

Cryptostegia grandiflora, as well as the related *C. madagascariensis* Boj., was described by Polhamus, Hill, and Elder (*12*),[4] and the morphology and anatomy of *C. grandiflora* were studied in detail by Artschwager (*3*). The plant grows rapidly from seed and produces a rounded shrub 6 feet or more in height. In addition to the usual foliage stems, long, upright, unbranched leaders 10 to 20 feet in length known as whips are produced in quantity. The whips will climb if a support is available, but in the absence of a support they ultimately bend over because of their own increasing weight and produce leafy lateral branches that are incorporated into the body of the plant.

Rubber from *Cryptostegia grandiflora* is obtained principally by bleeding the latex from the whips. The latex is contained in latex vessels found in the stems, roots, and leaves and even in the fruits. In the whips the latex vessels are present in the pith and the bark. Those of the pith, which number 300 to 800 and average about 25μ

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[3] Additional data on file in the Division of Rubber Plant Investigations, Plant Industry Station, Beltsville, Md.

[4] Italic numbers in parentheses refer to Literature Cited, p. 126.

in diameter, are formed early in the development of the whip and do not increase in number as the whip matures. The vessels of the bark, on the contrary, increase in number during the course of secondary growth. According to Whittenberger, Brice, and Copley (*19*), the proportionate amount of rubber in the bark increases as the whip matures. Thus, in whips 3 to 4 mm. in diameter 75 percent of the rubber was contained in the pith, whereas in those 17 mm. in diameter 74 percent was found in the bark.

MATERIALS AND METHODS

The plants used in these investigations were grown in the field at the laboratory experimental farm at Ciudad Victoria and at the experimental farm of the Mexican Government at Llera, Tamaulipas, Mexico. The seeds were germinated in nursery beds, and the seedlings were transplanted to the field when they had attained a height of 10 to 15 inches. Generally the plants were pruned to a height of 6 inches when transplanted and were planted in 3-acre plots in one of several spacings (1 by 1 to 3 by 12 feet). Cultural practices were then followed to maintain the plants in thrifty condition. An additional planting of about 3 acres of *Cryptostegia grandiflora* near Matamoros, Tamaulipas, Mexico, was also made available through the kindness of Ing. Eduardo Chavez. These plants were between 1 and 2 years old in 1943 and were growing in various spacings. They were irrigated as necessary and maintained in good growth.

Latex was usually obtained by whip bleeding. It was collected in cylindrical glass bottles about 3 cm. in diameter and 6 cm. in height. These were fitted with metal-rim screw caps which secured a rubber diaphragm over the top of the bottle. A hole 3 mm. in diameter allowed for the insertion of a cut whip through the diaphragm into the bottle. When successive bleedings of the same whip were made, the small plug of coagulated latex forming on the surface of the cut was removed by hand and weighed. This coagulate is referred to as "plug rubber," or "stem coagulate." "Total coagulate" includes the plug rubber as well as latex rubber. Latex coagulated by adding an equal volume of 95-percent ethyl alcohol is referred to as "coagulate," or "alcohol coagulate." For most yield studies the latex was alcohol-coagulated. Latex that was dried without addition of a coagulant is referred to as "total solids," or "latex total solids." The latex total solids were usually used in studies of a theoretical nature.

LATEX FLOW AND EXUDATION

FORCES INVOLVED IN LATEX FLOW

The force causing latex flow and exudation in *Hevea* was discussed by Arisz (*2*), Frey-Wyssling (*6*, *7*), Van Iterson (*10*), and others, and the literature on the subject was reviewed by Moyer (*11*). In general it may be stated that a latex vessel in an intact stem is under turgor pressure; the latex, for example, is under hydrostatic pressure. When a bleeding cut is made, the turgor pressure is reduced to zero at the point of incision while at a point some distance from the incision the original pressure still exists. The gradient in hydrostatic pressure thus set up on the latex is the driving force that causes latex flow and exudation from an incision. The force involved in latex flow is of

osmotic origin; the maximum pressure under which latex may be exuded would be expected to equal the osmotic pressure of the latex, or in general a force of several atmospheres.

Low humidity, drought, and other factors which set up moisture stress within the plant reduce the volume of flow and thus increase the concentration of the latex obtained. In 1943, for example, the spring and summer seasons were abnormally dry and high temperatures prevailed. During this time latex yields usually were higher on cool, cloudy days than on hot, sunny ones. Low relative humidity was directly correlated with low yields of latex, and winds caused further reductions in the volume of latex. Under these conditions the concentration of solids in the latex increased, probably because of loss of water from the latex vessels through water stress in the whip and the whole plant. Similar observations on latex flow in *Cryptostegia grandiflora* were made in Haiti by Curtis and Blondeau (*5*).

DILUTION OF LATEX DURING EXUDATION

When a *Cryptostegia grandiflora* whip is bled, the latex as it exudes becomes progressively more dilute. This response is illustrated in table 1, which is based on the amount of alcohol-coagulable material contained in successive exudations of latex from whips bled October 4, 1943, at Matamoros, Mexico. The latex collected during the first 15 seconds of exudation was four times as concentrated as that collected during the third and fourth minutes. The initial sample was twice as concentrated as the average for the entire bleeding.

TABLE 1.—*Average latex and concentration of coagulate in latex from whips of Cryptostegia grandiflora during exudation from single bleeding cuts, Matamoros, Mexico, Oct. 4, 1943*

[Each value from 50 whips]

Experiment and period after making cut	Latex per whip	Coagulate in latex	Experiment and period after making cut	Latex per whip	Coagulate in latex
Experiment 1:	*Milligrams*	*Percent*	Experiment 2:	*Milligrams*	*Percent*
0–15 seconds	44.5	28	0–15 seconds	13.0	32
16–30 seconds	19.7	24	16–30 seconds	4.9	29
31–60 seconds	39.7	16	31–60 seconds	41.2	21
61–120 seconds	80.9	10	61–120 seconds	61.5	11
121–240 seconds	78.2	6	121–240 seconds	57.9	9
Total or average	263.0	14	Total or average	178.5	15

This dilution of latex during exudation from *Cryptostegia grandiflora* is analogous to the dilution in *Hevea*, which was studied extensively by Arisz (*2*) and Frey-Wyssling (*6*, *7*). The dilution in *C. grandiflora* is, however, even more pronounced than that in *Hevea;* in a typical case described by Frey-Wyssling, the concentration of total solids in *Hevea* dropped from 39.1 to 33.2 percent during the course of the flow. The cause of the dilution was found by Frey-Wyssling to lie in the water relations of the latex vessel. Before bleeding, the vessel is under turgor pressure and in fact is in approximate water balance so that its suction force is small and its turgor pressure equals the osmotic pressure of its cell contents. When the bleeding cut is made, the turgor pressure is reduced to zero at the point

of the cut; therefore, the suction force of the vessel becomes equal to the osmotic pressure of the cell contents. Because of this, water is removed from the parenchymatous cells surrounding the vessels; as a result, with continued exudation the latex becomes progressively more dilute. The concentration of the latex collected in the initial period therefore gives a minimum figure for the concentration of the latex in the intact whip before bleeding.

A similar progressive diminution in latex concentration was found in whips bled daily for several days. Table 2 shows that in the course of five successive daily bleedings the concentration of total solids in the latex dropped in one experiment from approximately 22 percent at the first bleeding to 6.7 percent at the last. Similar behavior was repeatedly noted in other experiments.

TABLE 2.—*Average concentration of total solids in latex from mature whips of Cryptostegia grandiflora without growing tips bled on successive days, Ciudad Victoria, Mexico, Feb. 16–24, 1945*

[Each value from 30 whips]

Bleeding	Interval between bleedings	Total solids in latex from group—					
		1	2	3	4	5	6
	Hours	*Percent*	*Percent*	*Percent*	*Percent*	*Pcecent*	*Percent*
1		21.9	21.8	22.0	21.9	22.3	21.9
2	24	11.9	11.3	11.4	11.1	11.1	11.2
3	24	9.5	10.2	9.7	9.9	9.9	9.7
4	24	8.2	8.7	8.3	8.7	8.6	8.6
5	24				6.7	6.7	6.6

RECONCENTRATION OF TOTAL SOLIDS IN LATEX

Arisz (*2*) and Frey-Wyssling (*6, 7*) found that when the flow of *Hevea* latex ceases, because of the coagulation of the latex at the tapping cut, there is a reconcentration of the total solids in the latex still contained in the vessels. This is apparently due partly to the formation of new rubber in the latex and partly to osmotic removal of water from the diluted latex by the surrounding parenchymatous cells as the turgor of the vessels is reestablished.

The possible existence of a similar reconcentration in *Cryptostegia grandiflora* whips was investigated. At each of four or five daily bleedings half an inch of whip was removed from six comparable groups of whips. As stated previously, by this procedure the concentration of total solids in the latex was reduced from approximately 22 percent at the first bleeding to 6.7 percent at the fifth (table 2). For the next bleeding a 2½-inch piece of whip was removed at periods ranging from 0 to 96 hours after the preceding bleeding. The results were as follows:

Group:	*Period after 24-hour bleeding (hours)*	*Total solids in latex (percent)*	Group—Con.	*Period after 24-hour bleeding (hours)*	*Total solids in latex (percent)*
1	0	7.5	4	48	8.2
2	6	7.5	5	72	8.1
3	24	6.8	6	96	8.3

Even after 96 hours the concentration of total solids in the latex was far below that obtained at the first bleeding and was scarcely higher

than that obtained immediately after the fifth bleeding (table 2). This experiment offers no evidence of any considerable reconcentration of total solids in latex after bleeding in *C. grandiflora* whips.

In other experiments whips were bled at various periods after a single bleeding. The latex was collected at intervals up to 100 seconds so that the dilution during latex flow could also be studied. During the first bleeding a marked dilution occurred (table 3). When the second bleeding cut was made immediately after the initial one, the first latex exuded was more concentrated than the last obtained from the first cut. The reason for this may be that the latex exuded from the second cut comes partly from regions of the whip which did not supply latex during the initial flow. No reconcentration of the latex total solids was apparent after 10 or 50 minutes; in fact, latex collected 50 minutes after the first bleeding appeared to be even more dilute than that collected immediately after the first cut was made. This probably indicates that the dilution in *Cryptostegia grandiflora* (the passage of water from other tissues into the latex vessels) proceeds to some extent even after latex has ceased exuding. Furthermore, no reconcentration of the latex occurred in periods up to 24 hours after a cut was made (table 3).

TABLE 3.—*Average concentration of total solids in latex from succulent whips of Cryptostegia grandiflora with growing tips bled by making a second cut 1 inch below the initial at various intervals, Llera, Mexico, Apr. 10–12, 1945*

[Each value from 24 whips]

Experiment, date, and period after bleeding	Total solids in latex—							
	At initial bleeding	When a second cut was made after—						
		0 minute	10 minutes	50 minutes	1½ hours	4 hours	18 hours	24 hours
Experiment 1:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0–10 seconds	28.7	18.6	16.9	14.8				
11–20 seconds	17.5	17.9	16.3	9.3				
21–50 seconds	15.6	16.6	12.4	10.8				
51–100 seconds	13.9	16.1	11.8	7.5				
Experiment 2:								
0–10 seconds		13.2			10.9	10.6	11.9	11.0
11–20 seconds		12.6			9.3	9.9	10.5	10.7
21–50 seconds		9.7			9.1	8.2	8.5	9.8
51–100 seconds		6.8			6.5	7.8	6.1	

The data indicate that under the conditions of these experiments no considerable reconcentration of latex occurs within 10 minutes to 96 hours after bleeding. This is in contrast with *Hevea*, in which Arisz (*2*) found that a concentration of total solids equal to that of the initial latex after a single bleeding may be reestablished within approximately 12 hours.

LATEX MOVEMENT IN WHIPS AFTER A BLEEDING CUT

To study latex movement in whips after a bleeding cut had been made, latex vessels were internally blocked at various distances below the cut by freezing a short length of the whip with solid carbon

dioxide. Two pieces of solid carbon dioxide each having a volume of several cubic inches were held against the whip so that about a ½-inch length of stem was frozen. The stems were usually frozen solid within 30 seconds, but 60 to 90 seconds was ordinarily allowed to insure complete solidification. The points of freezing were at predetermined distances below the places where the cuts were to be made. The bleeding cuts were in the most apical living internode of mature whips whose terminal internodes had been killed by strong winds. Yield of latex was determined for 60 seconds after a cut had been made. Freezing did not appear to injure adjacent vessels, for when the frozen portion was cut off the remaining stump bled freely. When whips were frozen 40 inches below the apical cut, only a very slight diminution in yield resulted as compared with that from nonfrozen whips (table 4). When the distance below the bleeding cut was reduced to 20, 10, or 5 inches, further reductions in yield of latex occurred. From these data it may be calculated that the yield would have been reduced to half that from nonfrozen whips if the frozen block had been 18.5 inches below the bleeding cut. In other words, half of the latex yield from an initial bleeding cut in a whip originated within 18.5 inches of the cut.

TABLE 4.—*Latex flow after whips of Cryptostegia grandiflora had been frozen at various distances below the bleeding cuts made in upper third of the most apical living internode, Ciudad Victoria, Mexico, Feb. 17, 1945*

Distance between apical cut and frozen area	Plants treated	Latex flow per whip during first minute	
		At apical cut	Half inch below frozen area
	Number	*Drops*	*Drops*
Control (nonfrozen)	12	12.7	
40 inches	5	11.5	11.0
20 inches	6	6.8	9.5
10 inches	6	3.1	11.0
5 inches	7	1.1	11.6

In a second similar experiment the latex was collected at shorter time intervals than in the first to determine the length of whip contributing to the yield of latex during different periods of flow (table 5). At the various distances below the cut the effect of each block increased with time. Thus, although a block 20 inches below the cut

TABLE 5.—*Latex flow after whips of Cryptostegia grandiflora had been frozen at various distances below the bleeding cuts made in upper third of the most apical living internode, Ciudad Victoria, Mexico, Feb. 23, 1945*

Distance between apical cut and frozen area	Plants treated	Latex flow per whip during seconds indicated				
		0–10	11–20	21–40	41–60	0–60
	Number	*Drops*	*Drops*	*Drops*	*Drops*	*Drops*
Control (nonfrozen)	12	3.8	2.2	2.2	1.3	9.5
40 inches	12	3.6	2.1	2.1	1.2	9.0
20 inches	12	3.3	1.7	.8	.3	6.1
10 inches	12	1.6	.6	.3	.2	2.7
5 inches	12	.2	.3	.2	.1	.8

did not reduce latex flow during the first 10 seconds after the cut was made, thereafter it caused a marked reduction. As in the first experiment, the distances between the frozen block and the cut necessary for reduction of the latex flow to half that from the nonfrozen whips were calculated. The values obtained were as follows:

Period after making the bleeding cut:	*Distance necessary (inches)*
0–10 seconds	11. 6
11–20 seconds	14. 2
21–40 seconds	24. 2
41–60 seconds	26. 8
0–60 seconds	16. 2

These data indicate that the length of whip contributing to the yield of latex more than doubled during the 60 seconds of flow. Similar results in *Hevea* were obtained by Arisz (*2*) and Frey-Wyssling (*6*, *7*). The results may indicate that the lowering of the hydrostatic pressure in the vessels as a result of the incision is slowly extended along the whip.

In a third experiment the length of whip involved in latex flow was studied on a day when air temperature was low and there was a drying wind. Under these conditions the distance between block and bleeding cut necessary for reduction of the flow to half that of nonfrozen plants was only 11 inches, or considerably shorter than that found in the first two experiments. Under conditions of moisture stress latex flow is apparently confined to a shorter distance than under more favorable conditions.

These data indicate that after an initial whip bleeding cut 90 percent or more of the latex yield originates within 40 inches of the cut and that 50 percent originates within 10 to 20 inches, these distances varying according to prevailing environmental conditions. These conclusions were substantiated in a fourth experiment in which whips were subjected to two simultaneous bleeding cuts, an apical and a basal cut (table 6). This experiment, conducted on the same day as that reported in table 4, indicated, as in that experiment, that the yield of latex was reduced by half when each cut drew on only 18.5 inches of whip (p. 110).

These conclusions in respect to the length of whip involved in latex flow do not give exact information as to the distance that the latex actually exuded may have traveled within the whip. At least

TABLE 6.—*Latex flow from an apical cut in upper third of the most apical living internode of whips of Cryptostegia grandiflora made simultaneously with a basal cut, Ciudad Victoria, Mexico, Feb. 17, 1945*

Distance between apical and basal cuts	Plants tested	Latex flow during first minute	
		At apical cut	At basal cut
	Number	*Drops*	*Drops*
Control [1]	2	13.0	
80 inches	8	11.9	10.1
40 inches	6	7.3	8.0
20 inches	5	2.6	2.8
10 inches	2	1.0	.5

[1] See also the control in table 4 as the experiments were conducted on the same day.

two complications arise in determining these distances. The first is the dilution of exuding latex. Part of the latex exuded was not present in the latex vessels before the bleeding but entered the vessels as water after the bleeding cut was made. The volume of this water may be so great as to equal that of the original latex (table 1). Furthermore, water uptake at any given point in the vessel depends on the extent to which turgor pressure has been decreased at that point as a result of the bleeding cut. The second complication is based on the relation between distance from the incision and the turgor pressure of the latex vessel. The nature of this relation is not only unknown, but it also changes with time. It is apparent that until data are available on these points the actual length of whip from which the exuded latex originates will remain a matter of conjecture.

LATEX YIELD AND FACTORS AFFECTING IT

WHIP-BLEEDING YIELD ACCORDING TO INTERNODE

When whips are bled for the first time, the yield of latex varies according to the position of the internode which is cut. Therefore, the internodes of the whips to be cut were numbered basipetally, starting with the first internode that was more than an inch long. In several experiments the highest yielding internode was found to be the seventh from the topmost (table 7). In all cases yield was

TABLE 7.—*Latex and coagulate per whip of 1-year-old Cryptostegia grandiflora plants after 3 successive bleedings of various internodes followed in each case by an additional bleeding in the next internode, Matamoros, Mexico, 1943*

[Each value from 50 whips]

LATEX

Internode bled	Sept. 17	Sept. 18	Sept. 19	Sept. 20	Total for Sept. 17–19
	Grams	*Gram*	*Gram*	*Gram*	*Grams*
3	0.399	0.206	0.333	0.520	0.938
5	.592	.321	.388	.579	1.301
7	.828	.437	.455	.687	1.720
9	.988	.418	.460	.672	1.866
12	1.190	.427	.362	.562	1.979
15	.923	.169	.101	.162	1.193
18	.817	.046	.022	.058	.885

TOTAL COAGULATE

3	0.064	0.023	0.039	0.054	0.126
5	.092	.035	.045	.075	.172
7	.143	.048	.050	.089	.241
9	.135	.042	.045	.091	.222
12	.144	.042	.030	.046	.216
15	.127	.020	.007	.022	.154
18	.123	.007	.002	.004	.132

COAGULATE IN LATEX

	Percent	*Percent*	*Percent*	*Percent*	
3	15.7	10.3	10.3	9.1	
5	14.8	9.4	9.0	8.8	
7	16.7	8.9	7.3	7.6	
9	12.9	8.0	6.7	7.8	
12	10.8	7.4	6.6	6.5	
15	11.7	8.2	6.4	7.4	
18	11.5	12.0	7.4	1.0	

increased when after three bleedings within an internode the fourth bleeding cut was made in the next internode. This increase resulted mainly from an increase in volume of latex and not from an increase in the concentration of the coagulate in the latex. Apparently the nodes tend to restrict the flow of the latex. The percentage of coagulate in latex was always higher from the first bleeding in an internode than from subsequent bleeding cuts in the same internode. A comparison of decrease in yield of latex coagulate during three successive bleedings at 24-hour intervals within each of several internodes showed that the third internode decreased 39 percent, the fifth 51 percent, the seventh 65 percent, the ninth 67 percent, the twelfth 79 percent, the fifteenth 94 percent, and the eighteenth 98 percent. From the data it is apparent that the initial bleeding of a series should be made from the third to the sixth internodes, inclusive.

YIELD OF LATEX FROM VARIOUS BLEEDING SCHEDULES

Numerous experiments were carried out to determine the yield of latex obtainable from whips under various bleeding schedules. Bleedings were started at different times during the development of the whip, and the schedules were varied as to length of stem removed at each bleeding and interval between bleedings. Each bleeding schedule was usually carried out on samples of 50 to 125 whips. The data from a wide variety of such experiments are summarized in table 8.

The highest yields of alcohol coagulate, on a per whip basis, were obtained when bleedings were started in the third to sixth internode and 1 to 1½ inches of whip was removed at each subsequent cutting with an interval of 48 or 72 hours between bleedings. Almost as large yields per whip were obtained with four bleedings per internode followed by a 4- to 7-day interval between bleedings in each internode. Approximately 2 gm. of coagulate per whip was obtained by exhaustive bleeding of average-length whips on 1½- to 2-year-old plants under the conditions of these experiments.

It is of economic interest to consider the coagulate yield per bleeding. The highest yield calculated on this basis was obtained by a schedule of bleeding only once per internode with a 3- to 5-day lapse between cuttings. This schedule yielded a third to a half the amount obtained by exhaustive bleedings but necessitated only a fourth to a third as many bleedings. Estimates of rubber production by whip bleeding indicate that since approximately 40,000 whips per acre are produced annually under the conditions of these plantings, an annual potential yield of about 175 pounds of 85- to 90-percent rubber per acre would be indicated. It seems unlikely that more than two-thirds of the experimental yield could be obtained in commercial practice, so that a yield of about 120 pounds of 85- to 90-percent rubber per acre per year from 2-year-old plants of *Cryptostegia grandiflora* might be expected from a conservative whip-bleeding program.

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TABLE 8.—*Average yield of latex coagulate per bleeding of 5- to 7-inch internodes of Cryptostegia grandiflora on different bleeding schedules*

[Each value from 50 whips or more]

Period between successive bleedings of a single internode	Period between last bleeding of one internode and first bleeding of next	Whip length cut off	Internode bled first [1]	Total bleedings	Average yield per bleeding
	Hours	*Inches*		*Number*	*Milligrams*
½ hour		0.5	3	4	28
1 hour		.5	3	4	34
4 hours	20	.5	3	26	27
Do.[2]	120	.5	3	19	36
8 hours	96	[3] 2.3		12	19
24 hours		.5	3	26	31
Do.[2]	120	.5	3	14	51
24 hours		1.0	7	15	42
Do		[3] 7.0		14	50
Do		[3] 3.5		25	48
Do		[3] 1.8		25	46
Do	24	[3] 7.0		12	19
Do	96	[3] 2.3		12	22
Do	96	.5		12	21
Do	168	[3] 2.3		9	23
Do	240	[3] 2.3		9	26
48 hours		1.0	3	50	50
Do		1.0	6	47	49
Do		1.5	3	48	56
Do		1.5	6	45	57
Do		[3] 7.0		14	67
Do		[3] 3.5		25	62
Do		[3] 1.8		25	55
Do	48	.5	3	11	46
Do	72	.5	3	11	51
Do	120	.5	3	11	48
Do	168	.5	3	9	49
Do	240	.5	3	9	46
Do	120	1.0	3	12	64
Do	48			12	21
72 hours			3	10	33
Do		[3] 7.0		14	88
Do		[3] 3.5		25	65
Do		[3] 1.8		25	57
96 hours		1.0	3	8	39
120 hours		[3] 7.0		7	93

[1] Numbered basipetally.
[2] Only 4 bleedings per internode.
[3] Length cut off based on an average internode length of 7 inches.

EFFECT OF PREVIOUS BLEEDING ON LATEX YIELD

From the economic point of view it is of interest to know the influence of continued bleeding on yield of rubber. For this purpose an experiment was carried out to determine the effect of repeated bleedings on the amount of alcohol-coagulable material of latex. In this experiment one group of whips was bled four times per internode at 24-hour intervals. At each time a new internode was tapped, a fresh group of previously nontapped whips was bled in the corresponding internode for comparison. The data in table 9 show that the yield of coagulate decreased greatly with repeated tapping. At the time of the twenty-fifth bleeding (the first bleeding in the twelfth internode), for example, whips bled four times per internode at 24-hour intervals yielded a total coagulate of 0.041 gm. per whip. This low yield was due both to a low coagulate content of the latex (9 percent) and to a low yield of latex. The comparable previously nonbled whips yielded latex having 17 percent of alcohol coagulate and a total coagulate of 0.187 gm. per whip.

TABLE 9.—*Yields of total coagulate from repeatedly bled and once-bled 7- to 9-foot whips on cultivated, 15-month-old plants of Cryptostegia grandiflora*

[Bleedings every 24 hours, 4 per internode; each value from 50 whips]

Internode bled [1]	Single bleeding per whip	Successive bleedings of the same whip		
		First bleeding in internode	Total per internode	Cumulative yield
	Gram	*Gram*	*Gram*	*Grams*
6	0.110	0.133	0.323	0.323
7	.112	.059	.180	.503
8	.112	.058	.229	.732
9	.114	.055	.164	.896
10	.146	.041	.096	.992
11	.169	.048	.106	1.098
12	.187	.041		

[1] Numbered basipetally.

It would appear that the observed increase in latex yield and rubber content from whips bled at intervals of 72 hours as compared with those bled at shorter intervals resulted, in part at least, from the drawing of latex from farther down in the whip. These data agree with those reported under latex-movement studies.

COMPARISON OF LATEX OBTAINED BY BLEEDING WITH THE TOTAL IN THE WHIP

It would be of interest to know whether rubber is formed in the whip during the course of bleeding or whether exhaustive bleeding merely drains the whip, and perhaps even the plant, of rubber initially present. Data presented in the foregoing sections indicate that no detectable production of rubber takes place in the latex vessels during the usual bleeding intervals. Nevertheless, 539 mg. of rubber hydrocarbon was obtained per whip from 18 bleedings, whereas that originally present as shown by benzene extraction of comparable nonbled whips was only 240 mg. This difference would be further increased if the maximum number of bleedings possible, about 50, had been made.

The following experiment also indicates that the bleeding process itself may result in the development of a small amount of rubber not detectable by present methods of analysis. Sixteen groups of thirteen randomized whips each were selected and tagged on the plants. These whips were 2 to 3 feet long. The terminal 15 inches was cut off from the plants in six groups, and the cut surface of the excised part was immediately plunged into alcohol to prevent latex flow. A second lot of six groups was similarly treated except that they were bled after being cut from the plant in order to obtain all the latex possible. A third lot of four groups was bled just as the second lot was. The whips and latex of all lots were then dried and subjected to rubber analysis by the benzene-extraction method. In the second lot the dried latex rubber was analyzed separately from the whip, whereas in the third lot the latex rubber was blended with the whip for analysis as a check on recovery of the rubber.

The bled whips yielded significantly more total rubber than the nonbled controls and the yield increase approximated the amount of rubber obtained in the latex (table 10). This result, which was also obtained in two other experiments, might be taken to indicate that the extraction of rubber from the whip material was incomplete or that drying the whip before extraction destroyed the rubber. The first possibility seems remote, since exhaustive extraction of the woody whip material did not alter the result and also 100-percent rubber recovery was obtained in the third lot when rubber was added to the whip material before analysis. It appears that under some circumstances and with young whips, which were used in the present experiments, either the act of bleeding results in the formation of rubber or drying the whips destroys the rubber. This phenomenon further complicates studies on rubber formation in whips.

TABLE 10.—*Yield per whip of benzene-extractable material from cut-off terminal 15 inches of young 2- to 3-foot whips of Cryptostegia grandiflora bled and not bled after cutting, 1 to 3:30 p. m., Dec. 11, 1944*

[Latex and whips air-dried at 60° C.; each random sample average of 13 whips]

Yield from nonbled whips		Yield from bled whips when—					
		Latex and whip material separated				Latex and whip material together	
Sample No.	Whip material	Sample No.	Whip material	Latex	Total	Sample No.	Latex and whip material
	Milligrams		*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*
447	20.8	453	13.8	8.0	21.8	466	23.1
448	23.0	454	16.4	7.0	23.4	467	22.5
449	19.0	455	15.6	8.7	24.3	468	22.4
450	13.2	456	15.2	6.4	21.6	469	29.2
451	15.5	457	19.2	7.6	26.8		
452	17.3	458	20.2	7.6	27.8		
Average	18.1		16.7	7.6	24.3		24.3
Increase over nonbled whips					[1] 6.2		[2] 6.2

[1] Statistically significant at 1-percent level.
[2] Statistically significant between 2- and 3-percent levels.

RELATION OF LEAF RUBBER TO WHIP LATEX RUBBER

It has been mentioned previously that leaves of *Cryptostegia* contain both latex and cellular rubber. To determine whether there is a correlation between the rubber in the leaves and that in the latex, latex-rubber yield and leaf-rubber content were determined on a series of individual plants. Single whips on each of 10 individual plants were given 15 consecutive bleedings at 24-hour intervals. The latex was dried at 60° C. The yield from all the 15 bleedings was pooled for each whip and analyzed. Leaf samples from the same plants were similarly dried at 60° and analyzed. The rubber in the latex varied from 52.1 to 67.6 percent and that in the leaves from 1.39 to 3.02 percent. No correlation was found between the leaf rubber or resins and the whip latex-rubber content or yield. On the basis of present information, the absence of such correlations would necessitate latex

analyses and yield determinations on individual plants in connection with any breeding program for the development of high-yielding strains of plants with high-quality latex.

LATEX COMPOSITION AND FACTORS AFFECTING IT

CHEMICAL COMPOSITION OF LATEX

The latex of *Cryptostegia grandiflora* contains 5 to 25 percent of total solids, depending on the plant and on the circumstances under which the latex is collected. The solids are partly in the form of latex particles which are held in suspension in an aqueous serum. The latex particles were studied with the aid of the electron microscope by Hendricks, Wildman, and McMurdie (*9*), who showed that they vary in diameter from a few tenths of a micron to possibly 1μ and are approximately spherical. Vigorous mechanical agitation of the latex results in coagulation of the particles, which may then be completely separated from the serum by repeated washing.

The whole latex of a composite sample of *Cryptostegia grandiflora* collected at Llera, Mexico, on March 14, 1945, was found on fractionation to consist of 14 percent of mechanical coagulate and 86 percent of serum. The coagulate consisted of a small amount of protein, a substantial amount of acetone-soluble material (resins), and a very large amount of acetone-insoluble, benzene-soluble material, which is assumed to be rubber (table 11). The acetone-soluble, water-insoluble fraction would contain the triterpene ester studied by Hendricks and Wildman (*8*), who indicated that this compound may constitute as much as 90 percent of the fraction in latex of *C. madagascariensis* but that only a small amount is contained in the corresponding fraction of latex of *C. grandiflora*. Whether the resins are contained in the same latex particles as the rubber or in separate particles cannot be stated with certainty. The protein, however, probably represents material held on the surface of the particles, and as such it is to be regarded as a particle constituent. Tristram (*17*) indicated that in *Hevea* latex these particle-bound proteins are similar to the proteins of the latex serum, at least in amino acid composition.

TABLE 11.—*Latex constituents of a composite sample of Cryptostegia grandiflora collected at Llera, Mexico, Mar. 14, 1945*

Material	Solids in latex	Mechanical coagulate	Solids in serum
	Percent	*Percent*	*Percent*
Rubber (benzene-soluble)	57.1	[1] 84.4	
Resins associated with rubber	7.2	10.7	
Protein associated with rubber	2.1	[2] 3.1	
Acetone-soluble material:			
Citric acid	.3		0.8
Malic acid	.1		.3
Unknown serum resins	2.3		6.9
Phenol [3]	19.5		59.0
Potassium chloride	4.6		[4] 14.0
Amino acids	3.7		[5] 11.2
Protein of serum	3.0		[6] 9.1
Total	99.9	98.2	101.3

[1] After acetone extraction.
[2] Percent of N × 6.25.
[3] Derived from data of Stewart and Hummer (*15*).
[4] Based on analysis of comparable samples.
[5] α-Amino N × 7.
[6] Insoluble N × 6.25.

The serum of latex of *Cryptostegia grandiflora* may contain various amounts of total solids, which can be fully accounted for in terms of relatively well-known components. The representative latex of table 11 consisted of 7.9 percent of total solids and 92.1 percent of water. Of the solid material in the serum, 8 percent was acetone-soluble; this included small amounts of citric and malic acids determined by the methods of Pucher, Wakeman, and Vickery (*13*). The rest of the acetone-soluble material, however, was of unknown nature. The principal constituent of the serum solids was the substance described by Stewart and Hummer (*15*), an acetone-insoluble, ethyl-alcohol-soluble phenolic substance. This substance is of particular interest because large amounts of it were found in low-rubber latices and small amounts in high-rubber latices. That the substance is a phenol is indicated by its qualitative reactions, but its structure is not known. The serum further contained amino acids, potassium chloride, and protein. The amino acid content of *C. grandiflora* latex has a counterpart in the amino acids found in *Hevea* latex by Whitby and Greenberg (*18*) and Altman (*1*). None of the amino acids of *C. grandiflora* latex has as yet been isolated and identified, and their existence is necessarily based on the α-amino nitrogen content of the latex.

It was found that the proteins of *Cryptostegia grandiflora* latex contain a peroxidase. Freshly prepared serum was treated with an equal volume of saturated ammonium sulfate. The resulting precipitate was taken up in 0.05 M phosphate buffer, pH 5.5, the pH of the serum. In the presence of hydrogen peroxide this protein preparation was able to carry out rapid oxidation of *p*-cresol, guaiacol, β-naphthol, pyrogallol, *p*-phenylenediamine, and the latex phenolic substance of Stewart and Hummer (*15*). The reaction appears to be enzymatic, as indicated by inactivation of the peroxidase activity by prolonged heating at 100° C. It is of interest that the phenol, which is a normal constituent of the serum, is oxidized rapidly to an orange-colored product. In the freshly collected latex immediate and enzymatic darkening of the latex also takes place on the addition of hydrogen peroxide. This indicates that a significant amount of hydrogen peroxide does not accumulate in latex in the latex vessels.

Catalase activity was found to be very slight in latex of *Cryptostegia grandiflora*, but high in latex of *C. madagascariensis* and in that of the hybrid. Polyphenoloxidase and tyrosinase were lacking in the serum of *C. grandiflora*. Other enzymes were not investigated.

That enzymes occur among the serum proteins of other latices was indicated by Spence (*14*), who found peroxidase in *Hevea* latex. The latex of *Ficus* sp. is particularly rich in peroxidase (*16*), and proteolytic enzymes accumulate in other latices such as those of *Carica papaya* L. and *Asclepias* sp.

LATEX COMPOSITION AS AFFECTED BY BLEEDING

Analyses of latex total solids indicated that the ratio of rubber hydrocarbon to insolubles decreases in bleedings made within half an hour after an initial bleeding (table 12). This trend continues for two additional bleedings at ½-hour intervals. The composition of latex from a second bleeding 1 hour after the initial bleeding was practically the same as that from the third bleeding at ½-hour intervals. Appar-

ently latex composition is in part a function of lapsed time after the first bleeding as well as of the total number of bleedings.

TABLE 12.—*Average composition of pooled latex total solids in duplicate analyses of completely randomized samples from whips of Cryptostegia grandiflora bled successively at different intervals*

[Each value from 50 whips]

Component and period after first bleeding	Portion of total solids with indicated bleeding interval (hours)			
	½	1	4	48
Rubber hydrocarbon:	*Percent*	*Percent*	*Percent*	*Percent*
0 hour	61.6	62.8	61.9	61.3
½ hour	60.4			
1 hour	57.8	58.7		
1½ hours	54.1			
2 hours		54.5		
4 hours			54.1	
32 days				54.8
Insolubles:				
0 hour	21.7	21.1	23.4	26.1
½ hour	22.7			
1 hour	26.2	26.4		
1½ hours	30.8			
2 hours		30.5		
4 hours			31.0	
32 days				29.2
Resins:				
0 hour	16.7	16.1	14.7	12.6
½ hour	16.9			
1 hour	16.0	14.9		
1½ hours	15.1			
2 hours		15.0		
4 hours			14.9	
32 days				16.0

Latex was also obtained by a single initial bleeding from the upper, middle, and lower portions of whips. The yield from 50 samples of each portion was composited, dried at 60° C., and analyzed. There was 46.5 percent of rubber in the latex from the upper portion, 47.1 percent in that from the middle, and 52.6 percent in that from the lower. These data agree with the general observation for rubber-producing plants that old tissues usually have higher percentages of rubber than young ones.

Analyses of representative samples of air-dried latex collected after various consecutive bleedings in the course of different bleeding schedules are shown in table 13.

Latex obtained from the first bleeding of a whip always had a higher content of rubber hydrocarbon and a lower one of insolubles than that from subsequent bleedings. The latex total solids from the first bleeding usually contained 60 to 65 percent of rubber hydrocarbon and 21 to 24 percent of insolubles. Except in one of the schedules latex from the final bleeding was always lower in rubber hydrocarbon than from any of the intermediate bleedings. Rubber hydrocarbon content of the latex from the intermediate bleedings changed erratically. It was not possible to correlate these changes with any known factors such as weather, internode, or bleeding schedule. These data indicate that during the course of bleeding schedules through as much as 100 days enough synthesis of rubber hydrocarbon did not take place in the whip to equal that in the latex at the first bleeding.

TABLE 13.—*Composition of pooled representative samples of air-dried latex, from consecutive bleedings of 9- to 11-foot whips of Cryptostegia grandiflora, Llera, Mexico, first bleedings, June 22–25, 1944*

[Each value from 50 whips]

Sample	Bleeding schedule			Bleeding	Portion of latex	
	Whip length cut off	Bleeding interval	Internode bled first		Rubber hydrocarbon	Insolubles
	Inches	*Hours*			*Percent*	*Percent*
1	1	48	3	1	62.5	21.1
				15	52.8	31.3
				18	55.0	29.8
				19	54.4	26.2
				23	57.7	24.0
				50	43.9	28.6
2	1	72	3	10	54.8	30.3
3	1	96	3	8	56.2	30.3
4	1½	48	3	15	51.2	34.0
5	1	48	6	15	54.0	30.6
				18	55.4	30.8
				19	50.2	32.8
				23	49.6	32.1
6	1	[1] 48	3	4	56.2	28.6
				12	52.9	33.3
7	½	24	3	26	52.9	33.3
8	½	([2])		1	61.9	23.4
				2	54.1	31.0
				15	44.4	42.6
				16	45.9	40.8
				27	47.4	40.7
				28	48.0	40.2
				37	47.0	37.8
				38	45.2	38.6
				39	51.3	35.7
				40	47.2	38.0
				45	56.0	30.0
				46	51.2	36.3
9	½	([1 2])	3	5	51.2	35.6
				6	51.7	36.7
				9	46.5	41.2
				10	49.0	39.1
				11	57.5	31.6
				12	57.3	28.2
				13	50.9	34.0
				14	49.4	36.2
				15, 16	56.4	30.3
				17	50.1	32.3
10	½	([1 3])	3	13	54.8	30.5
				14	53.5	32.9
11	½	40	6	21	57.5	25.9
12	1½	48	3	21	61.5	19.1
				48	45.7	31.2

[1] Within each internode 4 bleedings were made as indicated, but there was a 5-day interval before bleeding in the next internode. [2] Twice daily. [3] Daily.

EFFECT OF TIME OF DAY ON LATEX COMPOSITION

Analyses of latex total solids from bleedings at either 6:15 a. m. or 3 p. m. from two groups of whips from October 23 to 28, 1944, are given in table 14. After the first three bleedings the schedules were reversed so that the group originally bled at 3 p. m. was bled at 6:15 a. m. and vice versa. On a percentage basis the rubber hydrocarbon was higher in the morning latex than in the afternoon, the difference being statistically significant at the 2- to 3-percent level. Insolubles were higher in the afternoon latex than in the morning, the difference being significant at the 1- to 2-percent level. In these computations the results for October 26 were omitted because the time intervals between successive bleedings of the morning and afternoon groups

differed owing to the previously mentioned change in the bleeding schedule. None of the differences in latex composition could be ascribed to sunlight intensities or other weather conditions.

TABLE 14.—*Average composition of latex total solids in duplicate analyses of latex from completely randomized 7- to 9-foot whips of Cryptostegia grandiflora bled at different times of day, Oct. 23–28, 1944*

[Each value from 50 whips]

Internode and date	Temperature range during preceding night	Relative humidity at 3 p. m.	Portion of latex total solids			
			Insolubles		Rubber hydrocarbon	
			6:15 a.m.	3 p. m.	6:15 a. m.	3 p. m.
Internode 5:	° F.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Oct. 23	80–58	63	22	26	61	58
Oct. 24	81–60	56	27	30	58	57
Oct. 25	80–55	58	37	42	50	45
Internode 6:						
Oct. 26	79–56	80	[1] 44	[2] 22	[1] 43	[2] 62
Oct. 27	74–62	76	40	43	45	42
Oct. 28	77–61	84	40	50	43	36

[1] Whips bled Oct. 25, 1944, at 3 p. m.
[2] Whips bled Oct. 25, 1944, at 6 : 15 a. m.

The evidence from two earlier experiments, together with the data in table 14, indicates that latex obtained after the night, or dark, period is higher in percentage of rubber hydrocarbon and lower in insolubles than that obtained after the day, or light, period. Such results, however, do not necessarily imply that insolubles are synthesized during the day and rubber hydrocarbon during the night, since they are on a percentage rather than actual-weight basis for these components.

LATEX YIELD AND COMPOSITION FROM FOLIATED AND DEFOLIATED PLANTS

Two groups of twenty 10-month-old plants were selected for uniformity of size. One group was then completely defoliated by hand. Three days later comparable-sized whips in both groups were bled for eight daily bleedings with four bleedings per internode. The latex from the first four bleedings was alcohol-coagulated, whereas that from the second four was air-dried.

The foliated plants consistently yielded more coagulate than the defoliated ones (table 15), the difference amounting to about 12 percent for the first bleeding but increasing to about 40 percent for all of the remaining bleedings. Yield of latex was greater from the defoliated plants for the first two bleedings; thereafter the foliated plants yielded more latex, which had a higher percentage of rubber. Such results may indicate that the leaves are essential for production of coagulate in latex, as well as for maintaining the flow of latex during a number of bleedings. The greater flow from the defoliated plants for the first two bleedings was probably a direct effect of the removal of the leaves. In this respect, the artificially defoliated plants were similar to naturally defoliated ones studied in western Mexico.

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TABLE 15.—*Coagulate per whip from consecutive daily bleedings of foliated and defoliated plants of Cryptostegia grandiflora*

[Each value from 20 plants]

Condition of plants and bleeding No.	Kind of latex	Latex	Latex coagulate	Stem coagulate	Total coagulate	Coagulate in latex
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*
Foliated:						
1	Alcohol-coagulated	213	34.2	24.3	58.5	15.9
2	do	168	7.5	5.2	12.7	4.5
3	do	162	10.5	2.0	12.5	6.5
4	do	175	5.0	1.7	6.7	2.8
5	Air-dried	182	11.7	3.5	15.2	6.4
6	do	152	10.7	1.0	11.7	7.0
7	do	138	8.7	.8	9.5	6.3
8	do	133	8.7	.3	9.0	6.5
Defoliated:						
1	Alcohol-coagulated	247	35.2	16.3	51.5	14.2
2	do	176	6.7	1.0	7.7	3.9
3	do	132	7.5	.7	8.2	5.7
4	do	127	3.5	.4	3.9	2.8
5	Air-dried	167	7.0	2.7	9.7	4.2
6	do	132	8.2	.6	8.8	6.2
7	do	126	6.5	.5	7.0	5.2
8	do	128	8.0	.2	8.2	6.2

To obtain additional information on the effect of leaves on latex the defoliated plants studied were divided into four different categories of foliation, and a group of the original nondefoliated plants were retained as controls. A summary of the results from periodic bleedings of these groups is shown in table 16.

TABLE 16.—*Latex total solids per whip from plants of Cryptostegia grandiflora under various conditions of foliation, beginning with an original defoliation on January 27, 1944*

[Each value from 5 plants bled in intact internodes]

Condition of plant	Yield on bleeding date indicated					Total yield
	Feb. 24	Mar. 3 [1]	Mar. 15	Mar. 28		
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*	*Milligrams*
Never defoliated (control)	32	42	13	14	8.8	101
With regained foliage	18	41	13	19	12.2	91
Continuously defoliated	22	32	20	9	5.1	83
With defoliated whip but regained foliage on rest of plant	20	37	17	11	7.6	85
Continuously defoliated except for regained foliage on whip	24	41	20	17	11.7	102

[1] Total yield from 3 bleedings at 24-hour intervals in same internode.

It was found that the lower yielding whips were those on the plants which had been continuously defoliated or those whips that were the only parts continuously defoliated. The whips on plants allowed to grow new leaves after the initial defoliation and those on plants on which only the whip was allowed to grow new leaves yielded more nearly the same amount of coagulate as whips on plants which had never been defoliated. Similarly, the whips yielding latex containing lower percentages of total solids were those on plants on which the entire plant or the whip alone had been continuously defoliated. The evidence indicates that leaves are necessary to maintain the

total solids in latex and suggests in addition that the only leaves directly involved in whip bleeding are those on the whip being bled. At the time of year when this experiment was carried out (February and March) there were no apparent effects of defoliation on the relative amounts of rubber hydrocarbon, resins, and insolubles in the latex.

COMPOSITION OF LATEX FROM ROOTS, SEED, AND YOUNG SEEDLINGS

Latex collected from the taproots of over fifty 1-year-old plants of *Cryptostegia grandiflora* contained 30.7 percent of rubber hydrocarbon, 31.7 percent of insolubles, and 37.6 percent of resins. In most cases, the exudation was so slight that scraping the cut surfaces with a knife was necessary in order to collect sufficient latex for analysis. Judging from its composition latex from roots may have a fundamentally different physiology from that of above-ground parts of the plant, since the percentages of rubber hydrocarbon and insolubles in the root latex do not fall along the established regression line for these components (*15*).

To obtain sufficient seedling latex for analysis between 500 and 700 seedlings were grown on paper towels kept moist with a complete nutrient solution. When the seedlings were 3 weeks old and about 2 inches high their cotyledonary leaves were cut off and the small droplet of latex exuding from the stump was collected on a glass slide and dried at 60° C. The yield of latex total solids from all of the seedlings amounted to only 17.9 mg. By a bromination analysis this was found to contain 3.4 percent of rubber hydrocarbon and 42.5 percent of insolubles. Like those of the root latex, these percentages do not agree with the correlation between rubber hydrocarbon and insolubles found in latex from older plants. These data indicate that rubber hydrocarbon occurs in very young seedlings even before they have developed their true foliage leaves and so open the possibility of studying rubber formation in seedlings.

It was not possible to detect rubber hydrocarbon in ungerminated seeds. Approximately 200 gm. of seed was extracted with benzene, but bromination of the extract failed to yield any rubber tetrabromide, which it is expected it would have yielded had the extract contained rubber hydrocarbon.

COMPOSITION OF LATEX FROM INTERSPECIFIC RECIPROCAL GRAFTS

Analyses were made of latex from various combinations of bud and cleft grafts between *Cryptostegia grandiflora*, *C. madagascariensis*, and the F_1 hybrid. These grafts were made between October 15 and November 15, 1943, at Ciudad Victoria, Mexico. Latex was obtained approximately a year later by bleeding the whips or in the absence of whips by bleeding branches less than 5 mm. in diameter. When shoots (suckers) from the stock also had been produced, they were bled separately from the scion. On some plants 2 different scions had been grafted to the same stock, 1 of the scions being of the same species as the stock. The various combinations of grafts and the analytical results of 15 of the 31 grafts tested are presented in table 17.

TABLE 17.—*Composition of total solids from latex obtained by whip or stem bleeding of various reciprocal graft combinations between Cryptostegia grandiflora, C. madagascariensis, and the F_1 hybrid, Ciudad Victoria, Mexico*

Plant	Species of stock	Species of scion	Growth of stock bled	Growth of graft bled	Portion of latex		
					Rubber hydrocarbon	Insolubles	Undetermined
					Percent	*Percent*	*Percent*
15	*grandiflora*	*madagascariensis*		Whiplike stem	1.7	40.7	57.6
14	do	do		Foliage stems	2.0	35.9	62.1
			Sucker whip		52.8	30.7	16.5
16	do	do		Whiplike stem	1.7	41.7	56.6
			Sucker whip		56.0	28.6	15.4
28	*madagascariensis*	*grandiflora*		Whips	53.5	31.2	15.3
25	do	do		do	45.9	38.9	15.2
			Foliage stem of sucker		1.5	42.5	56.0
27	do	do		Whip	49.8	34.5	15.7
			Young foliage stem of sucker		.7	38.6	60.7
17	*grandiflora*	Hybrid		Foliage stem	28.5	36.1	35.4
			Sucker whip		55.0	29.1	15.9
20	do	do		Foliage stem	27.9	36.8	35.3
			Foliage stem of sucker		55.6	29.0	15.4
11	do	do		Foliage stems	28.0	40.2	31.8
			Whip		54.6	33.7	11.7
24	*madagascariensis*	do		Foliage stem	45.6	28.4	26.0
9	Hybrid	*grandiflora*		Whip	51.8	31.3	16.9
			Sucker whip		36.3	29.3	34.4
22	do	do		Whip	51.1	30.7	18.2
			Foliage stem of sucker		36.0	35.0	29.0
31	do	*madagascariensis*		Foliage stem	1.5	44.0	54.5
			Sucker whip		35.3	26.3	38.4
32	do	do		Foliage stem	1.4	40.7	57.9
			Sucker whip		38.0	27.2	34.8
33	do	do		Foliage stem	1.3	43.7	55.0
			Sucker whip		36.9	25.1	38.0

The data indicate that rootstock has no determining influence on the percentage of rubber hydrocarbon and insolubles in the latex from the scion. Likewise, there was no evidence that the scion influenced the percentages of these substances in latex from the stock. It was not possible to determine whether the stock affected the yield from the scion because of a lack of whips on the scions comparable with those on nongrafted plants.

It appears from these results that the role of the roots in the formation of rubber or of insolubles in the latex is an indirect one at best. This directs attention to the role of the above-ground parts of the plant in the synthesis of these two fractions. The possibility of grafting scions of high yield but low quality upon high-quality stocks to improve the quality of the latex from the scion does not seem promising.

ATTEMPTS AT RUBBER FORMATION IN VITRO

To determine whether latex serum from *Cryptostegia grandiflora* latex prepared as described might contain enzymes or substances capable of bringing about rubber formation in vitro at the expense of substances already in the latex or of materials added to the latex which might conceivably act as precursors of rubber, the following preparations from *C. grandiflora* were tested as sources of enzymes in attempting rubber formation in vitro: Serum containing some rubber, rubber-free serum, purified serum proteins, whole-leaf cytoplasm, purified leaf-cytoplasm proteins, and chloroplasts from leaves of *C. grandiflora*.

The following substances, which have been suggested as theoretically possible rubber precursors, were tested in concentration series from 1 to 10 mg. per milliliter as additions to the preparations just named in attempting rubber formation in vitro: None (formation of rubber by the preparation alone), citral, β-methyl crotonaldehyde, tiglic acid (sodium salt), isovaleric acid (sodium salt), isoleucine, isovaleraldehyde, tiglaldehyde, phenol isolated from *Cryptostegia grandiflora* insolubles, triterpenol from *C. madagascariensis* latex, and various ones of those previously listed plus hydrogen peroxide.

In each case the formation of rubber after overnight incubation at room temperature was estimated by drying down the whole reaction mixture and extracting it with benzene, in some cases after a preliminary extraction with alcohol. Then 5 ml. of the benzene extract was pipetted into an equal volume of alcohol, and the turbidity of the solution was measured in a Coleman photofluorometer.

In no case was any suggestion of rubber formation in vitro found.

These experiments included many of the possible rubber precursors suggested in the literature (*4*), as well as their closely related derivatives; in addition three possible sources of rubber-forming enzymes—namely, latex-serum proteins, leaf-cytoplasm proteins, and chloroplastic proteins—were used. The results were uniformly completely negative. Whether current theories of rubber formation are in error or an improper experimental approach was used cannot be stated.

SUMMARY AND CONCLUSIONS

Studies on latex physiology of *Cryptostegia grandiflora* have led to the following conclusions.

Latex flow from whips is influenced by relative humidity, wind, and temperature. The effects of these various environmental factors are interrelated. These factors appear to influence latex flow by affecting the water relations of the plant. Latex as it flows from a cut whip becomes progressively more dilute. In the course of exhaustive bleeding of a whip, sufficient rubber synthesis does not occur to maintain the concentration of rubber as found in the latex obtained in the initial bleeding. No reconcentration of the total solids in latex occurs in periods of 10 minutes to 96 hours after a bleeding cut is made. Of the total movement of latex in the first minute after a bleeding cut is made, 90 percent or more takes place within 40 inches of the bleeding cut.

Successive whip bleedings decrease both the yield of latex and the percentage of rubber in the latex. The total rubber yields by exhaustive whip bleeding may approach as much as 3 gm. per whip for 2- to 3-year-old plants. For 1½- to 2-year-old plants, in general, the yield is about 2 gm. A significantly higher yield of rubber is obtained by both bleeding and extracting a whip than by either process alone. No correlation between leaf rubber and the rubber content or yield of the whip latex was detected.

Latex can be coagulated by mechanical agitation; 98.2 percent of the coagulate can be accounted for by rubber (benzene-soluble), resins (acetone-soluble), and proteins. The remaining serum consists of 92.1 percent of water and 7.9 percent of solids. Of these solids all but 6.9 percent can be accounted for on the basis of known chemical fractions.

Whip latex obtained in the morning is higher in percentage of rubber and lower in percentage of insolubles than that obtained in the afternoon. The presence of foliage leaves on the whip being bled repeatedly is necessary for maintenance of the total-solids content of the latex. No rubber hydrocarbon can be detected in ungerminated seeds. The latex total solids from 3-week-old seedlings contain significant amounts of rubber. The solids of latex obtained from roots of mature plants contain as much as 30.7 percent of rubber. The rootstock does not influence the composition of the latex of the scion.

Tests with several possible enzyme preparations with several possible rubber-precursor substrates gave no indication of rubber synthesis in vitro.

LITERATURE CITED

(1) Altman, R. F. A.
1940. organic analysis of hevealatex. iv. amino-acids. Arch. v. Rubbercult. Nederland. Indië 24: [647]–654. [In Dutch. English summary, p. 653.]

(2) Arisz, W. H.
1928. physiologie van het tappen. [physiology of tapping.] Arch. v. Rubbercult. Nederland. Indië 12: [220]–241, illus.

(3) Artschwager, E.
1946. contribution to the morphology and anatomy of cryptostegia (cryptostegia grandiflora). U. S. Dept. Agr. Tech. Bul. 915, 40 pp., illus.

(4) Bonner, J., and Galston, A. W.
1947. The physiology and biochemistry of rubber formation in plants. Bot. Rev. 13:543–596.
(5) Curtis, J. T., and Blondeau, R.
1946. Influence of time of day on latex flow from cryptostegia grandiflora. Amer. Jour. Bot. 33: 264–270, illus.
(6) Frey-Wyssling, A.
1932. Investigations on the dilution reaction and the movement of the latex of hevea brasiliensis during tapping. Arch. v. Rubbercult. Nederland. Indië 16: [285]–327, illus.
(7) ———
1933. Der milchsafterguss von hevea brasiliensis als blutungserscheinung. Ein beitrag zur druckstromtheorie. Jahrb. f. Wiss. Bot. 77: [560]–626, illus.
(8) Hendricks, S. B., and Wildman, S. G.
1946. A triterpene ester isolated from cryptostegia madagascariensis latex. Arch. Biochem. 10: 157–162.
(9) ——— Wildman, S. G., and McMurdie, H. F.
1944. Morphology of latex particles as shown by electron micrographs. India Rubber World 110: 297–300, illus.
(10) Iterson, G. van, Jr.
1936–37. Rubber symposium at Delft. X—hevea brasiliensis as a producer of rubber. India-Rubber Jour. 92 (26): 3–9 (869)–(875), 1936; 93 (1): 11–18 (23)–(30); 93 (2): 10–15 (60)–(65), illus., 1937.
(11) Moyer, L. S.
1937. Recent advances in the physiology of latex. Bot. Rev. 3: 522–544.
(12) Polhamus, L. G., Hill, H. H., and Elder, J. A.
1934. The rubber content of two species of cryptostegia and of an interspecific hybrid in florida. U. S. Dept. Agr. Tech. Bul. 457, 23 pp., illus.
(13) Pucher, G. W., Wakeman, A. J., and Vickery, H. B.
1941. Organic acids in plant tissues: modifications of analytical methods. Indus. and Engin. Chem., Analyt. Ed. 13: 244–246.
(14) Spence, D.
1908. On the presence of oxidases in india-rubber, with a theory in regard to their function in the latex. Biochem. Jour. 3: 165–181.
(15) Stewart, W. S., and Hummer, R. W.
1945. Inverse correlation between rubber hydrocarbon and a crystalline fraction isolated from latex of cryptostegia grandiflora. Bot. Gaz. 106: 333–340, illus.
(16) Sumner, J., and Somers, G. F.
1943. Chemistry and methods of enzymes. 365 pp., illus. New York.
(17) Tristram, G. R.
1941. The proteins of hevea brasiliensis. 2. analysis of a product isolated from crepe rubber. Biochem. Jour. 35: 413–416.
(18) Whitby, G. S., and Greenberg, H.
1941. The isolation of amino-acids from rubber latex. Biochem. Jour. 35: 640–649.
(19) Whittenberger, R. T., Brice, B. A., and Copley, M. J.
1945. Distribution of rubber in cryptostegia as a factor in its recovery. India Rubber World 112: 319–323, illus.

SUSCEPTIBILITY OF GUAYULE TO VERTICILLIUM WILT AND INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON DEVELOPMENT OF INFECTION [1]

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INTRODUCTION

In the winter and spring of 1942 and 1943 extensive plantings of guayule (*Parthenium argentatum* A. Gray) were made in California. Although several diseases of guayule had already been described,[3] the seriousness of some of them, especially verticillium wilt, caused by *Verticillium albo-atrum* Reinke and Berth., was not fully realized. The results of later surveys on the occurrence of verticillium wilt and the losses caused by it have been reported,[4] and the disease has been discussed by Campbell and Presley (*5*).[5] This paper records the results of tests to determine (1) the susceptibility of eight commercial strains of guayule to verticillium wilt and (2) the influence of soil temperature and moisture on the development of infection. An abstract of the findings of the preliminary experiments has been published (*18*).

MATERIALS AND METHODS

The eight commercial strains [6] of guayule most commonly planted from 1942 to 1945 under the emergency rubber project program were grown at various places in California to test their reaction to verticillium wilt under different environmental conditions. The effect of soil temperature on the disease in strain 109 was studied in the greenhouse at Salinas, and the effect of soil moisture on the disease

[1] Received for publication August 1, 1947.

[2] The author is indebted to W. A. Campbell, formerly pathologist, special guayule research project, for making valuable suggestions and for facilitating the work.

[3] CAMPBELL, W. A., LEACH, L. D., PRESLEY, J. T., and SNYDER, W. C. SOME DISEASES OF GUAYULE IN CALIFORNIA. U. S. Bur. Plant Indus., Plant Dis. Rptr. 27: 63–66. 1943. [Processed.]

[4] SCHNEIDER, H. SURVEYS AND OBSERVATIONS ON VERTICILLUM WILT OF GUAYULE IN CALIFORNIA FROM 1943 TO 1945. U. S. Bur. Plant Indus., Soils, and Agr. Engin., Plant Dis. Rptr. 29: 615–617. 1945. [Processed.]

[5] Italic numbers in parentheses refer to Literature Cited, p. 142.

[6] Selected originally by W. C. McCallum, formerly employed by the International Rubber Co. and the Emergency Rubber Project (U. S. Forest Service).

when affecting strain 593 was studied in field plots at Shafter. The details of the experiments are presented in the sections in which the results are given.

The data were analyzed by Snedecor's analysis-of-variance methods (*19*). Because the data involved the number of diseased plants or of plants remaining healthy out of a given number observed, the values were changed to percentages, which were transformed to angles (*19, table 16.8*). When none or all of the plants observed had the particular attribute the original values were adjusted before they were changed to percentages by assuming, for instance, that ¼ of 1 plant remained healthy when all were diseased and that only 9¾ of 10 plants remained healthy when 10 were observed to be healthy (Bartlett's correction (*1*)). The *F* test was applied to the mean squares obtained.

In some instances analyses were also run without either Bartlett's correction or transformation of the data. The *F* values obtained, but not used herein, were not appreciably different from those computed after the transformations. From the mean squares obtained from the analyses the mean differences required for significance between means of treatments were calculated. Although according to some authorities on statistical methods these values theoretically may not be as sensitive as those from transformed data, they can be used with the means from the original data rather than with means of angles, which are rather abstract.

SUSCEPTIBILITY OF COMMERCIAL STRAINS OF GUAYULE

The eight strains of guayule commonly planted may be loosely grouped into four main types on the basis of the external morphological characteristics which they have in common. Strain 109 comprises one type; 111 a second; 130, 406, and 593 a third; and 405, 407, and 416 a fourth. The number of chromosomes (*2*) and the sizes, dry weights, and resin and rubber content of these strains (*8*) have been studied. These strains reproduce mostly by apomixis; therefore, for practical purposes each may be considered an asexual clone (*7, 14*). For several reasons the strains used were not pure (*8*)[7]—the seed fields had not been rogued, there was some variation in reproduction (*2, 14*), and there may have been some mixing of seed. Strain 109 had the largest number of offtype plants. The typical plant of this strain was most susceptible to verticillium wilt; therefore, the injury reported is an underestimate for the typical plant of strain 109. Since the cause of variation was not known when the work was begun, it did not seem advisable to attempt to sort out the typical plants.

In early summer of 1943 parts of the approximately 30,000 acres of guayule planted in different parts of California were showing the effects of verticillium wilt. One of the most heavily infected fields, located near Shafter, was planted to strain 109, but through error two rows of strain 405 plants were also planted in this field (fig. 1). Most of the typical plants of strain 109 were severely stunted or killed, but the majority of those of strain 405 and many of the offtype plants of strain 109 showed little disease, although they were infected.

[7] Nursery plants or seeds were obtained from the Emergency Rubber Project.

Many plants of strains 406, 593, and 130 in other fields showed symptoms ranging from slight injury to killing at the same time, but usually plants of strains 405 and 407 were less severely affected. During the summer of 1943 in the warm interior valleys many diseased plants of all strains recovered. Later when the cool temperatures of fall were again favorable for the development of verticillium wilt, only the plants of strain 109 showed external symptoms.

Near Salinas a preliminary test of six commercial strains of guayule was made in a nursery-type variety planting (five randomized blocks) on soil infested with *Verticillium albo-atrum* and nematodes (*Heterodera marioni* (Cornu) Goodey). At the end of the growing season the taproots were cut to see what percentage had dark-brown discoloration of the wood and were therefore presumably

FIGURE 1.—Field of guayule strain 109 in which through error two rows of strain 405 had been planted, Shafter, Calif., July 1943. Note that plants of strain 109 were severely affected with verticillium wilt whereas those of strain 405 (center) were only slightly affected.

infected by *Verticillium*. The results were as follows: Strain 109, 74 percent; strain 593, 66 percent; strain 406, 56 percent; strain 130, 53 percent; strain 416, 47 percent; and strain 407, 42 percent. According to the *F* test the difference in susceptibility among strains was highly significant.

In the winter of 1943–44 field tests were begun in four localities. The designs were randomized blocks or Latin squares. Details of the experiments follow:

The Greenfield experiment was located in the middle of the Salinas Valley where soil temperatures favorable for activity of *Verticillium* prevailed all summer. The plots were planted December 31, 1943, and final data were taken September 18, 1944. As *Verticillium* was not uniformly distributed through this field, the differences between means are small and the differences required between means for significance are large (table 1).

Salinas experiment 1 was located in the lower Salinas Valley where soil temperatures favorable for activity of *Verticillium* prevailed all summer. The plots were planted June 26, 1944, and harvested March 27, 1945.

TABLE 1.—*Average number of guayule plants out of 8 of each strain affected in varying degrees with verticillium wilt in field-type plantings, California* [1]

Location of planting	Degree of injury of plants	Plants of type and strain								Amount for significance at—		F test
		Type 1 (109)	Type 2 (111)	Type 3			Type 4			5-percent level	1-percent level	
				130	406	593	405	407	416			
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*			
Greenfield [2]	Killed	2.6	2.2	0.9	0.1	0.3	0.3	0.4	0.1			
	Severely and moderately affected	1.7	1.6	2.4	2.1	3.7	1.0	.6	.6			
	Slightly affected and healthy	3.7	4.3	4.7	5.7	4.0	6.7	7.0	7.3	1.8	2.4	**
Salinas (trial 2) [3]	Killed	1.8		.7	.1	.4	.4	.1	.1			
	Severely and moderately affected	4.7		4.0	4.5	4.7	1.2	.9	.3			
	Slightly affected and healthy	1.5		3.3	3.3	2.8	6.4	7.0	7.5	1.0	1.3	**
Salinas (trial 1) [4]	Killed			.3	.1	.1	.3	.1				
	Severely and moderately affected			2.5	2.4	2.1	.6	.4				
	Slightly affected and healthy			5.2	5.5	5.8	7.1	7.5		.9	1.2	**
Shafter [5]	Killed	0	0	0	0	0	0	.1	.1			
	Severely and moderately affected	3.1	1.6	.1	0	.3	.8	.4	.3			
	Slightly affected and healthy	4.9	6.4	7.9	8.0	7.8	7.3	7.5	7.6	.9	1.2	**

**Highly significant.
[1] For details concerning the plots see p. 131.
[2] Each value is an average for 7 plots.
[3] Each value is an average for 12 plots.
[4] Each value is an average for 15 plots.
[5] Each value is an average for 8 plots.

Salinas experiment 2 was adjacent to Salinas experiment 1. The plots were planted April 1, 1944, and harvested August 21, 1944.

The Shafter experiment was located near Shafter. The plots were planted November 25, 1943, and the final data were taken February 27, 1945. Summer air temperatures were unfavorable for the disease. (See fig. 3.) Since irrigating tends to cool the soil, the plots were irrigated weekly in an attempt to keep the soil temperature within a range favorable for verticillium wilt. This experiment and another in the same locality were established on land on which guayule had been severely diseased the previous year. Although spring temperatures in both years were favorable for disease activity, symptoms were not severe in either plot the second year, indicating possibly that *Verticillium* does not build up in soil planted to guayule. Injury was not so severe in this locality as in others where temperatures were more favorable for the fungus.

In each plot eight plants chosen at random were graded according to injury from *Verticillium*. The symptoms were observed monthly; at the end of the experiments the plants were pulled and the taproots were cut to see whether there were any dark-brown discolorations in the wood. In two of the experiments the rubber yields also were determined. The data in tables 1 and 2 indicate that degree of injury and reduction in yields were correlated. It had been found previously [8] that plant size was reduced by verticillium wilt but that on the dry-weight basis the percentage of rubber was the same in injured plants as in healthy ones.

TABLE 2.—*Rubber yield per acre of guayule strains grown on Verticillium-infested soil, California*

[8 plants harvested per plot]

Strain	Yield per acre at—	
	Greenfield [1]	Salinas (trial 1) [2]
	Pounds	*Pounds*
109	25	----
111	25	----
130	35	42
406	35	42
593	34	44
405	43	54
407	43	57
416	42	----
Difference required for significance:		
5-percent level	14.4	9.5
1-percent level	----	12.6

[1] Mean of 7 replicates of 8 plants of each strain. The differences are not significant according to the *F* test.

[2] Mean of 15 replicates of 8 plants of each strain. The differences are highly significant according to the *F* test.

It may be concluded that strain 109 is very susceptible to verticillium wilt; strains 130, 406, and 593 are moderately affected; and strains 405, 407, and 416 are affected least, though occasional plants may be killed. Differences in susceptibility between the last two groups did not occur in the Shafter trial probably owing to unfavorable summer temperatures. Strains within morphologically similar groups of plants were for the most part similar in resistance to the disease. It is interesting to note that strains which yield best in the absence of the disease (*8*) yield least when the disease is present.

[8] See footnote 4, p. 129.

SOIL-TEMPERATURE STUDIES

The effect of soil temperature on verticillium wilt has received considerable attention. Ludbrook (*12*) tested cultures from *Verticillium*-diseased plants from various parts of the world, including several from California. He found in culture that one group of fungi which he designated as *Verticillium albo-atrum* R. and B. had a lower maximum temperature for growth than a microsclerotium-producing group which he designated as *V. dahliae* Klebahn. He found that growth of *V. albo-atrum* was greatly retarded at 82° F. and none occurred at 86°. *V. dahliae* grew well at 82° and all cultures of it made some growth at 86°. The results obtained when plants were grown in infested soil at controlled temperatures indicated that these fungi had the same maximum temperatures in plants as in cultures. McKeen (*13*) found that the microsclerotium-producing fungus designated as *V. albo-atrum* produced its maximum damage in tomatoes at a soil temperature of 75° and became inactive at about 86°. Williams (*20*) recently found that in culture *V. dahliae* is favored by slightly higher temperatures than *V. albo-atrum*.

The microsclerotium-producing fungus here designated as *Verticillium albo-atrum* segregated into microsclerotium- and non-microsclerotium-producing cultures on single sporing, as isolates from other diseased agricultural crop plants had previously been found to do (*15*). Two isolates were tested—one from near Salinas, where moderate temperatures prevail, and the other from near Shafter, where temperatures are presumably too high to favor the development of *V. albo-atrum* in the summer.

The soil in which these isolates were tested was maintained at constant temperatures ranging from 51° to 105° F. in cans suspended in tanks of water like those described by Campbell and Presley (*4*). The cans were 18 inches high. The lower 4 inches was filled with sand; above this was 8 inches of sandy loam. About 200 cc. of oat cultures per can was mixed with the soil.[9] No sterile oats were used in the control cans. A space of 6 inches was left above the soil surface to provide warm air over the soil, and the top of the soil was covered with glass wool to minimize temperature changes at the soil surface. Glass tubes were inserted on the sides of the cans so that any accumulation of water at the bottom of the cans could be detected. Two plants of strain 109 which had been grown from seed in 2-inch pots were planted in each can. Three cans containing soil infested with the fungus from near Shafter, three containing soil infested with the fungus from Salinas, and two control cans containing uninfested soil were used at each temperature. Soil temperatures were taken morning and night. The averages of all the readings, together with the dry weights of the plants determined after 9 weeks, are given in figure 2. The weights shown are the average weights of plants per can from two cans selected at random from each set of three cans. The check data are the average weights of plants per can from the two control cans. The plants made practically no growth at 51° and 105°, and apparently the infected plants were stunted at temperatures between 72° and 79°. At temperatures of 85° and above no infection occurred, but the oat medium seemed to affect fertility.

[9] The medium for the cultures was prepared by boiling whole oats.

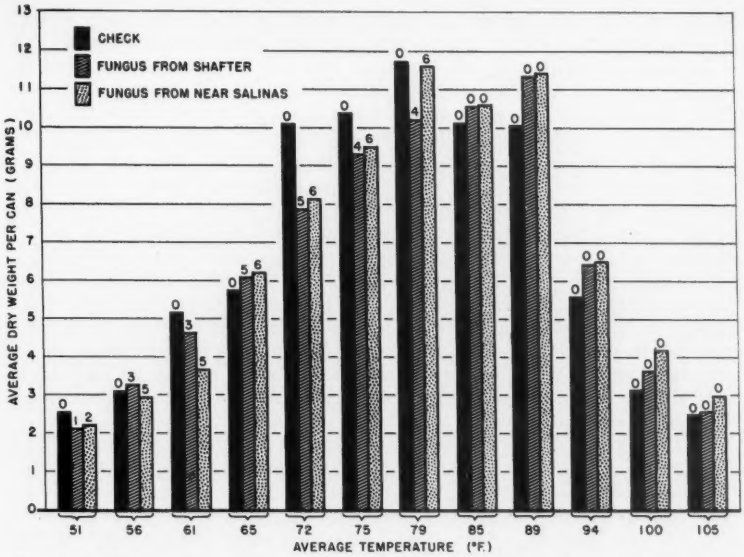

FIGURE 2.—Dry weight of plants grown in infested soil and in clean soil at various temperatures. The numbers above the bars indicate how many of the six plants in each series were infected.

Summer temperatures in the Salinas Valley are considered optimum for growth of *Verticillium*, but those in Kern County are believed to be unfavorable for its development during the warm summer months. For ease of comparison air and soil temperatures for Salinas and air temperature for Bakersfield, which is near Shafter and has about the same temperature, are presented graphically in figure 3. Soil temperatures for Shafter are shown in figure 4.

EFFECTS OF SOIL MOISTURE AT SHAFTER

The study of the relation between soil moisture and verticillium wilt brings up two questions: (1) What is the relation between soil

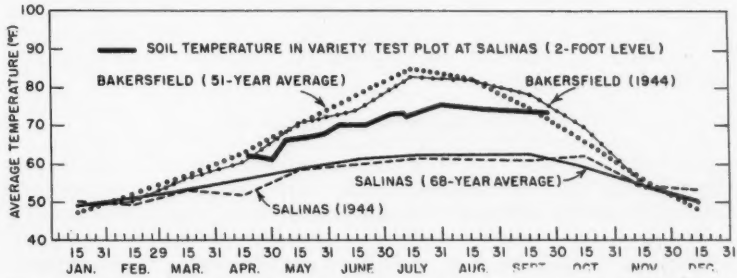

FIGURE 3.—Monthly means of each day's mean air temperature for Bakersfield, Calif., which is near Shafter and has about the same temperature, and for Salinas, Calif., and soil temperatures at the 2-foot level at Salinas. (Air-temperature data from the U. S. Weather Bureau.)

moisture and the number of infections by *Verticillium*? (2) Which moisture tensions favor the disease once the plants are infected?

Literature published before 1931 on soil moisture as related to verticillium wilt was reviewed by Rudolph (*17*). McKeen (*13*) found that when *Verticillium* inoculum was placed in dry soil and fallowed for 2 months before cropping, the fungus took longer to attack the plants than when inoculum was so placed in wet soil. Rada (*16*) reported that infection of young cotton plants of one of the three varieties tested did not occur so soon when the first irrigation was delayed for 2 months after planting as when it was delayed only 1 month. All subsequent irrigations were the same for both treatments. Infections were determined by observing the discolorations in the vascular bundles of leaf petioles. As the treatments were not replicated, the observed delay in infection may well have been due to lighter infestation of the soil. Final counts determined by the presence of discoloration of the vascular tissue of the stem at the ground level indicated no differences due to treatments. No reference was made to rainfall or soil moisture.

Ludbrook (*12*), in greenhouse tests with eggplants, attempted to maintain soil at various percentages of the moisture-holding capacity (a condition of soil moisture brought about by empirical methods described by Riker and Riker [10] and somewhat above field capacity) by weighing the pots at intervals and adding enough water to bring the ratio of water to soil to some desired percentage. In such cases, as Hendrickson and Veihmeyer (*9*) have found, added water wets only a portion of the soil. For this reason no actual difference in water tension between Ludbrook's treatments occurred, but the top layers of soil in all treatments were maintained at the same degree of wetting. Therefore his conclusion that soil moistures between 45 and 95 percent of the moisture-holding capacity had no appreciable effect on verticillium wilt of eggplant except at 82.4° F. (at which a lengthening of the incubation period and a reduction in severity occurred in his highest two soil-moisture treatments) appears to be invalid. Isaac (*11*) also studied the effect of soil moisture on the disease in sainfoin plants by adding enough water at intervals to bring the soil to various percentages of the water-saturation point. He gave no data on the field capacity or wilting point of the soil. His results showed that "an increase in the water content of the soil decreases the incidence of disease" (*11*, *p. 33*). Apparently the soil moistures which Ludbrook and Isaac found to be adverse to the disease were above field capacity and would not be found in the field in well-drained soils for any length of time after irrigations. Perhaps the effect produced was caused by limitation of the aeration.

1943 IRRIGATION EXPERIMENT

In August 1943 an experiment was set up in a field moderately affected with wilt near Shafter, Calif. The following arbitrary irrigation treatments were used: (1) Irrigation every week (water applied

[10] RIKER, A. J., and RIKER, R. S. INTRODUCTION TO RESEARCH ON PLANT DISEASES—A GUIDE TO THE PRINCIPLES AND PRACTICE FOR STUDYING VARIOUS PLANT-DISEASE PROBLEMS. 117 pp., illus. St. Louis, Chicago, etc. 1936. [Processed.]

for 30 minutes after it reached the ends of the furrows); (2) irrigation every 2 weeks (water applied for 1 hour after it reached the ends of the furrows); (3) irrigation every 4 weeks (water applied for 2 hours after it reached the ends of the furrows); and (4) no irrigation. The last field irrigations before part of the field was taken over for the experimental plots was about July 1. The experimental irrigations were begun on August 6 when water was applied in treatments 1, 2, and 3. The last irrigation was made on October 22. The experiment was set up on a well-drained Hesperia sandy loam with the wilting point ranging from 7 to 10 percent and the field capacity from 15 to 20 percent depending on the location and depth of the soil. The treatments were replicated in 5 randomized blocks. For each plot 20 plants were selected—5 healthy, 5 slightly affected, 5 moderately affected, and 5 severely affected. The condition and growth of these plants were observed each month.

Soil-moisture determinations by the oven-dry method were made every 2 weeks on the day before irrigation to the 4-foot level for each plot. No determinations were made immediately after the irrigations, but penetration as a result of short irrigations in this type of soil when it was at the wilting point was 1 to 2 feet. The average soil-moisture conditions for the first 4 feet in the plots follow.

Of the plots irrigated at 1-week intervals that in block A remained above 15 percent at all levels. Between irrigations plots in the other blocks did not dry quite down to the wilting point in the upper 2 feet, and after the middle of October the upper 2 feet of soil dried to only about 15 percent. The water did not penetrate beyond the 2-foot level in these four plots, and the soil was near the wilting point at the lower depths.

In all except three of the plots irrigated at 2-week intervals the soil approached the wilting point when sampled. In these three plots the moisture was slightly above the wilting point between the 2- and 4-foot levels during August and the first half of September.

In the plots irrigated at 4-week intervals the soil approached the wilting point within 2 weeks after irrigation at all levels except the 2- to 4-foot levels during August and the first half of September.

All the dry plots were at the wilting point after August 20 except those in three of the blocks in which the soil at the 2- to 4-foot levels did not reach the wilting point until about the end of September.

On April 1, 1944, for the plants which were apparently healthy on August 6, 1943, the number of infections was determined by cutting the taproots. On the plots irrigated every week 77 percent of the plants were infected by *Verticillium;* on those irrigated every 2 weeks, 62 percent; on those irrigated every 4 weeks, 42 percent; and on those left dry, 8 percent. The *F* test indicates highly significant differences. In the dry plot occasional plants that showed recovery from previous infection by *Verticillium*, but that were not detected by cutting branches on August 6, were recorded as healthy. On one plot two plants and in each of four others one plant had died from causes other than infection by *Verticillium.*

From the results obtained from these plots the following general conclusions seemed justified: Frequent irrigations favored infection by *Verticillium.* On the average, moderately and severely affected plants

remained so in dry plots, but such plants showed about the same degree of improvement in each of the three irrigated treatments. Since temperatures were unfavorable for the disease during August and September, recovery was probably more from the severe injury that resulted from the spring epidemic than from active verticilliosis. Slightly affected plants responded like healthy ones regardless of the type of treatment. In dry plots growth as indicated by plant spread and dry weight was greatly reduced; rubber content, however, was increased.

1944 IRRIGATION EXPERIMENT

In 1944 an attempt was made to prevent infection by keeping the upper layers of soil—where *Verticillium* was assumed to occur in the greatest concentration—near the wilting point at times when temperatures were favorable for infection. Four treatments replicated five times were set up on a different part of the field that had been used for the 1943 experiment. The ground chosen was heavily infested. Guayule plants set out the previous year were removed, and a new planting of strain 593 was made. The ground was wet to the 7-foot level by irrigations before and during planting.

Treatment 1 consisted of irrigation of the soil for 2 hours each week. Plaster of paris blocks described by Bouyoucos and Mick (*3*) for determining soil moisture were placed at 1-, 3-, and 5-foot depths. Treatment 2 consisted of irrigation after the 1-foot level of soil had been at the wilting point for 1 week. The plots were irrigated until the profile was wet to 6 feet. Since irrigating was done only once a week, the plots were irrigated as soon as possible after the 1-week period had elapsed, but not exactly at the end of 1 week. Plaster of paris blocks were placed at 1-, 2-, 3-, 5-, and 7-foot depths. Treatment 3 was the same as treatment 2 except that no irrigations were given when the temperature of the soil was below 80° F. at the 18-inch level. In treatment 4 no irrigating was done after the plants became established. In treatments 3 and 4 plaster of paris blocks were placed at the same levels as in treatment 2.

Readings of 60,000- to 75,000-ohm resistance in the plaster of paris block were considered to indicate that the soil was at the wilting point (*3*).

The plots were planted during the second week of March in rows 28 inches apart and with plants 20 inches apart in the rows. It was hard to get the plants to grow, and nongrowers were replanted on March 30, May 17, and June 9. The replants were watered by hand.

Spring Infections

On May 18, 15 vigorous plants, each having at least 3 neighbors, were selected and staked in each plot for further observation. For final analysis 10 of these not affected by other causes were selected at random. It was soon obvious that the water was not being withdrawn from the soil rapidly enough to bring about the desired differences in treatments during the period of favorable temperatures in spring. Therefore, the original design was modified for the spring period by irrigating every 2 weeks instead of every week

in treatment 2. As indicated in figure 4, there was little difference between the treatments in the number of infections that occurred, but the number of infections was significantly greater in plots irrigated weekly than in the plots of the two treatments that had not received water since the treatments began.

Injury to plants in the spring was not severe, possibly because the inoculum potential did not build up in the soil when it was cropped to guayule the previous year. Temperatures were very favorable for *Verticillium* in the spring of 1943 and 1944, but in the plants chosen for observation the symptoms were slight except in a few that were moderately affected. There were no differences between treatments.

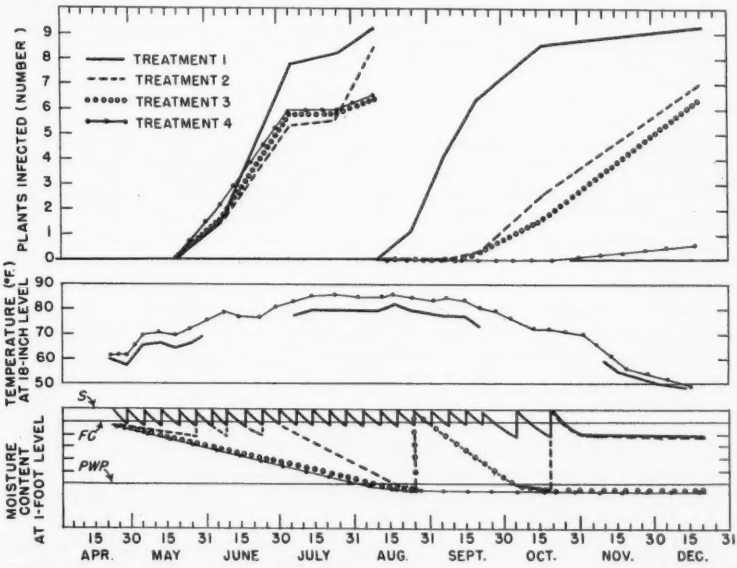
PLANTS INFECTED (NUMBER)
TREATMENT 1
TREATMENT 2
TREATMENT 3
TREATMENT 4
TEMPERATURE (°F.) AT 18-INCH LEVEL
MOISTURE CONTENT AT 1-FOOT LEVEL
S
FC
PWP
15 30 15 31 15 30 15 31 15 31 15 30 15 31 15 30 15 31
APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC.

FIGURE 4.—Relation of soil temperature and soil moisture to verticillium wilt of 10 plants chosen as healthy on May 18, 1944, and of 10 chosen as healthy on August 9 in 5 randomized blocks given various irrigation treatments, Shafter, Calif. For the August 10 readings at the 5-percent level 1.7 plants were required for significant differences between means of the May plants, and for the December 20 readings 4.2 plants were required for significant differences between means of the August plants; at the 1-percent level 2.2 and 5.9 plants, respectively, were required. *FC*, field capacity (approximated); *PWP*, permanent wilting point; *S*, saturation point.

Yields from the plants selected on May 18 in the various plots were determined on December 20 (table 3). Withholding irrigations caused a highly significant increase in rubber content and a highly significant decrease in dry weight. The yields were lowered by withholding water, but not significantly so. This inverse relation of rubber production and shrub weight was also found by Hunter and Kelley (*10*) in healthy plants on a Delano sandy loam near Shafter.

TABLE 3.—*Effect of irrigation treatments on rubber content, dry weight, and yield of guayule plants in 5 randomized blocks collected Dec. 20, 1944, Shafter, Calif.*

[Each value based on a composite sample of 10 plants from each plot]

Treatment No.	Rubber content [1]	Average dry weight per plant [1]	Rubber yield per acre [2]
	Percent	*Grams*	*Pounds*
1	3.00	153.9	113.7
2	3.01	148.4	110.8
3	3.46	125.2	106.2
4	4.47	84.6	92.9
Difference required for significance:			
5-percent level	.39	22.1	16.7
1-percent level	.55	31.0	23.4

[1] Differences highly significant according to *F* test.
[2] Differences not significant according to *F* test.

FALL INFECTIONS

On August 9, 10 apparently healthy plants (mostly replants) of equal size and having 4 neighbors were chosen in each plot and staked. Temporary spring treatments were discontinued on July 7 and those originally outlined were begun. The occurrence of infection as related to temperature and moisture are shown in figure 4. For the December 20 data plants were pulled and the taproots were cut to determine infection. For other dates the branches were cut on plants exhibiting possible top symptoms. Moisture data for the 1-foot level are presented in figure 4. The rate at which the lower levels reached the wilting point was related to the treatments. In treatment 2 in August the wilting point was reached at the 2-foot level within 10 days after it was reached at the 1-foot level in 1 replicate; the irrigation was made before the wilting point was reached at the 2-foot level in the other 2 replicates in which plaster of paris blocks were used. Only 1 replicate had reached the wilting point at the 2-foot level at the time of the October irrigation. In treatment 3 only 1 replicate had reached the wilting point at the 2-foot level at the time of the August 25 irrigation. The same replicate was at the wilting point again at the 2-foot level on October 17, and the other 2 had reached the wilting point at the 2-foot level by November 10. One replicate had reached the wilting point at the 3-foot level by December 20. In treatment 4 the wilting point at the various depths was reached on the following dates: Replicate A: 1-foot level, August 24; 2-foot level, August 24; 3-foot level, October 17; 5-foot level, December 20. Replicate C: 1-foot level, July 24; 2-foot level, August 26; 3-foot level, September 7; 5-foot level, October 17; 7-foot level, not reached. Replicate E: 1-foot level, July 24; 2-foot level, August 24; 3-foot level, September 20; 5- and 7-foot levels, not reached.

Continued low soil-moisture tension appeared to favor infection (treatment 1), but when the upper layers of soil, in which the fungus is probably most extensively distributed, were maintained at the wilting point few infections occurred (treatment 4). Frequent irrigations lowered the soil temperature; as a result, infection occurred during the summer in treatment 1. Drying the upper layers of soil to the wilting point between irrigations in treatments 2 and 3 had no significant effect on the final readings as compared with those in

treatment 1, but it did delay infection until the temperature began to moderate in the latter part of September. Infections occurred in treatment 3 when the soil moisture was somewhat below the field capacity but above the wilting point.

DISCUSSION

Because of the nature of guayule there are several possible methods of reducing losses from verticillium wilt. Guayule differs from most agricultural crops in that it is a desert plant, which can withstand severe drought. In fact, yields of rubber (pounds per acre) may be as high, depending on soil type, when plants are grown under high soil-moisture tension as when grown under low tension (*10*). The reason is that the percentage of rubber formed increases with an increase of moisture tension.

In a climate like that of Shafter, Calif., after plants are once established in the field, some degree of control may possibly be effected by allowing the upper 2 or 3 feet of soil to remain at the wilting point as long as possible, especially when temperatures are favorable for the growth of *Verticillium*. However, while plants are being established this method of control is not possible. There are several reasons for this. In the first place, it is necessary to maintain low soil-moisture tension to establish plants (*6*), and it takes several months after plants begin growth for them to develop tops and root systems large enough to dry the soil to the wilting point. Moreover, the temperature must be sufficiently high to cause the plants to begin growth but not so high as to cause rapid drying of the top 6 inches of soil. Such moderate temperatures also favor the growth of *Verticillium*. However, some plants escape infection during this period of establishment, possibly because their root systems are not sufficiently extensive to reach the fungus. Many other plants are only slightly affected and recover. That woody plants recover from verticillium wilt is well known. Recovery occurs in both guayule and trees such as apricot, in which rings of discolored wood are found only in occasional years. Each discolored ring is presumably the result of a new infection. In guayule the cell walls of infected wood turn dark brown, and the vessels become filled with a brown amorphous substance. This material may cause the death or isolation of the fungus in affected vessels. There are also some indications that multiple infections are more serious than a single infection. The only decided reduction in infection in the experiment described here occurred when the soil was maintained at the wilting point.

Once plants are established and are large enough to withdraw water from the soil rapidly, certain irrigation practices may be beneficial in preventing further infections. In the interior valleys of California where high summer temperatures are unfavorable for the growth of the fungus, it seems desirable to irrigate only in summer provided there is no danger from phytophthora root rot or drowning, which occurs when irrigation, high temperatures, and certain other conditions are combined (*5*). When winter irrigating is done, the water may not be completely withdrawn from the upper layers of soil until new growth begins in spring. Then it is gradually withdrawn. Since the fungus also becomes active at this time, the maximum number of infections occurs. Plants of strain 593 beginning their second year's

growth in the field have shown severe discoloration of the taproots under these conditions. Top symptoms, however, were not noticed in this strain, though stunting of the plants no doubt occurred.[11]

The strain tests have shown that when the fields to be used are known to be infested with *Verticillium*, it is essential that very susceptible strains be avoided.

SUMMARY

Experiments conducted in California from 1942 to 1945 indicated that the commercial strains of guayule differ in susceptibility to *Verticillium albo-atrum*. Plants of strain 109 are very susceptible to verticillium wilt and may be killed at any age. Less extensive observations and tests indicate that strain 111 is similar to 109 in susceptibility. Strains 405, 407, and 416 are the most resistant, but occasional plants may be killed by verticillium wilt when young. Strains 130, 406, and 593 are intermediate in resistance.

Experiments in constant-temperature tanks indicated that *V. albo-atrum* becomes inactive at soil temperatures between 80° and 85° F. Irrigation experiments at Shafter, Calif., in field trials seem to show that this fungus is active in soil at all moisture levels above the wilting point. Maintaining the upper soil horizons at the wilting point prevents infection.

LITERATURE CITED

(1) Bartleti, M. S.
1947. The use of transformations. Biometrics Bul. 3: 39–52.

(2) Bergner, A. D.
1946. Polyploidy and aneuploidy in guayule. U. S. Dept. Agr. Tech. Bul. 918, 36 pp., illus.

(3) Bouyoucos, G. J., and Mick, A. H.
1940. An electrical resistance method for the continuous measurement of soil moisture under field conditions. Mich. Agr. Expt. Sta. Tech. Bul. 172, 38 pp., illus.

(4) Campbell, W. A., and Presley, J. T.
1945. Design for constant-temperature tanks. Phytopathology 35: 213–216, illus.

(5) ——— and Presley, J. T.
1946. Diseases of cultivated guayule and their control. U. S. Dept. Agr. Cir. 749, 42 pp., illus.

(6) Erickson, L. C.
1945. The water factor in transplanting guayule. Amer. Jour. Bot. 32: 634–643, illus.

(7) Esau, K.
1946. Morphology of reproduction in guayule and certain other species of parthenium. Hilgardia 17: [61]–120, illus.

(8) Federer, W. T.
1946. Variability of certain seed, seedling, and young-plant characters of guayule. U. S. Dept. Agr. Tech. Bul. 919, 25 pp.

(9) Hendrickson, A. H., and Veihmeyer, F. J.
1941. Moisture distribution in soil in containers. Plant Physiol. 16: 821–826, illus.

(10) Hunter, A. S., and Kelley, O. J.
1946. The growth and rubber content of guayule as affected by variations in soil moisture stresses. Amer. Soc. Agron. Jour. 38: 118–134, illus.

(11) Isaac, I.
1946. Verticillium wilt of sainfoin. Ann. Appl. Biol. 33: 28–34, illus.

[11] See footnote 4, p. 129.

(12) LUDBROOK, W. V.
1933. PATHOGENICITY AND ENVIRONAL STUDIES ON VERTICILLIUM HADROMYCOSIS. Phytopathology 23: 117–154, illus.
(13) MCKEEN, C. D.
1943. A STUDY OF SOME FACTORS AFFECTING THE PATHOGENICITY OF VERTICILLIUM ALBO-ATRUM R. & B. Canad. Jour. Res., Sect. C, 21: [95]–117, illus.
(14) POWERS, L., and ROLLINS, R. C.
1945. REPRODUCTION AND POLLINATION STUDIES ON GUAYULE, PARTHENIUM ARGENTATUM GRAY AND P. INCANUM H. B. K. Amer. Soc. Agron. Jour. 37: 96–112.
(15) PRESLEY, J. T.
1941. SALTANTS FROM A MONOSPORIC CULTURE OF VERTICILLIUM ALBO-ATRUM. Phytopathology 31: 1135–1139, illus.
(16) [RADA, G. G.]
1939. INFLUENCIA DEL RIEGO EN EL "WILT" DEL ALGODONERO. Lima, Peru, Estac. Expt. Agr. de La Molina, Mem. 11a: 233–240, illus.
(17) RUDOLPH, B. A.
1931. VERTICILLIUM HADROMYCOSIS. Hilgardia 5: [197]–360, illus.
(18) SCHNEIDER, H.
1944. VERTICILLIUM WILT OF GUAYULE. (Abstract) Phytopathology 34: 936.
(19) SNEDECOR, G. W.
1946. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 4, 485 pp., illus. Ames, Iowa.
(20) WILLIAMS, P. H.
1944. VERTICILLIUM WILT. [Cheshunt] Expt. and Res. Sta. Ann. Rpt. (1943) 29: 29–33.

SUCROSE, DEXTROSE, AND LEVULOSE CONTENT OF SOME DOMESTIC VARIETIES OF SORGO AT DIFFERENT STAGES OF MATURITY[1]

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INTRODUCTION

Berthelot and Trannoy [2] studied the sucrose, dextrose, and levulose content of the juices of one variety of sorgo at eight stages of growth selected at about 14 days apart. They found that the dextrose content was consistently greater than the levulose content and both reached a minimum at the stage of maturity at which the sucrose content reached a maximum.

Willaman, West, Spriestersbach, and Holm,[3] after a study of three varieties of sorgo, reported that the amount of dextrose exceeded the amount of levulose throughout the growth of the plant, and they remarked that this characteristic of sorgo is different from that of most plant juices in which levulose generally is found to exceed dextrose. In general their results agree with those of Berthelot and Trannoy.

Ventre, Byall, and Walton,[4] in a study of the crystallization characteristics of sorgo sirups, noted that sirups made from immature canes and from the lower portions of the stalks crystallized dextrose, whereas the crystallization of sucrose occurred in sirups made from the upper portions of the stalk and from more mature canes. These facts suggested that by separation of the cane into two parts and working each separately one part would furnish on crystallization a source of sucrose and the other a source of dextrose. These facts further suggested that some varieties of sorgo might contain a higher content of dextrose than the four varieties used by Ventre and his associates.

MATERIALS AND METHODS

To supplement the previous studies and to obtain data on the principal varieties of sorgo (*Sorghum vulgare* Pers.) grown in the United States, the work described in this paper was carried out in cooperation with the Division of Sugar Plant Investigations, Bureau of Plant In-

[1] Received for publication August 15, 1947. This report formed part of a paper entitled, "The Sucrose, Dextrose, and Levulose Content of Some Sorgo Varieties With a Discussion of Factors Involving Their Utilization for Sugar Production," presented at the meeting of the American Chemical Society at Baltimore, Md., April 1939.

[2] BERTHELOT, D., and TRANNOY, R. SUR LA TENEUR EN SUCRE, DU SORGHO AUX DIVERS STADES DE SA VEGETATION. [Paris] Acad. des Sci. Compt. Rend. 166: 824–827. 1918.

[3] WILLAMAN, J. J., WEST, R. M., SPRIESTERSBACH, D. O., and HOLM, G. E. NOTES ON THE COMPOSITION OF THE SORGHUM PLANT. Jour. Agr. Res. 18: 1–31, illus. 1919.

[4] VENTRE, E. K., BYALL, S., and WALTON, C. F., JR. JELLYING AND CRYSTALLIZATION OF SIRUPS MADE FROM DIFFERENT PARTS OF THE SORGO STALK AT DIFFERENT STAGES OF MATURITY. Jour. Agr. Res. 59: 139–150. 1939.

dustry, Soils, and Agricultural Engineering, United States Department of Agriculture. Thirty-four known varieties of sorgo were grown at the field station of that division near Meridian, Miss. The whole sorgo cane was cut and stripped, the seed heads were removed, and the cane was milled as soon as possible on a power-driven farm sirup mill which gave about 60 percent juice extraction. As far as it was possible to do so, each variety was studied in the three principal stages of maturity as denoted by the seeds being in the milk, dough-to-ripe, and dead-ripe stage. The juices expressed after being strained through a 300-mesh sieve were subjected to the following analytical determinations.

The concentration of dissolved solids or specific gravity in degrees Brix was determined on the freshly strained juice by a Brix hydrometer calibrated at 20° C.

Sucrose polarization, *total reducing sugars*, *dextrose*, and *levulose*.—A portion of each lot of freshly strained juice equivalent to five normal weights (26 gm.) was transferred to a 250-cc. volumetric flask, 12 cc. of a saturated neutral lead acetate solution was added, and the solution was made up to the mark and filtered.

(a) *Reducing sugars*.—To 50 cc. of the filtrate in a 200-cc. volumetric flask, 1 cc. of McAllep-Cook's solution, containing 7 gm. of disodium phosphate and 3 gm. of potassium oxalate to 100 cc., was added for each gram of total dissolved solids taken; the solution was made up to the mark and filtered; and the total reducing sugars were determined by the Lane-Eynon method.[5]

(b) *Polarization*.—Determinations were made by reading a deleaded portion of the filtrate in a jacketed 400-mm. tube in a saccharimeter at 20° C.

(c) *Sucrose* (*Clerget*).—Determinations were made on the filtrate according to the official method of the Association of Official Agricultural Chemists [6] with invertase as the inverting agent.

(d) *Sucrose, by copper reduction*.—The total quantity of reducing sugars was determined by the Lane-Eynon method [7] on the inverted solutions from the sucrose (Clerget) determination. Sucrose was calculated from the difference between the total reducing sugars as invert sugar determined before, and total reducing sugars as invert sugar determined after, inversion with invertase.

(e) *Levulose*.—The invertase-inverted solution from the sucrose (Clerget) determination was polarized in a metal-jacketed, 400-mm. polariscope tube at a temperature interval of about 60° C., and the total levulose content of the solution calculated from the factors determined by Jackson and Mathews.[8] The total levulose content of the inverted solution of the juice, corrected for the amount of levulose formed in the inversion of sucrose determined from the sucrose by copper reduction, was taken as the original levulose content of the juice.

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[5] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. . . . Ed. 5, 757 pp., illus. Washington, D. C., 1940. (See pp. 498–499.)

[6] See pp. 491–493 of reference cited in footnote 5.

[7] See pp. 498–499 and 683–684 of reference cited in footnote 5.

[8] JACKSON, R. F., and MATHEWS, J. A. SOME PHYSICAL PROPERTIES OF LEVULOSE AND ITS ESTIMATION BY COPPER REDUCTION METHODS. [U. S.] Natl. Bur. Standards Jour. Res. 8: 403–444, illus. 1932.

Dextrose was determined by calculation from the foregoing determinations as follows: Total reducing sugars as invert sugar on uninverted juice (a) minus the levulose content of the original juice as determined under (e) calculated as invert sugar gives the dextrose content of the original juice in terms of invert sugar and is translated into dextrose by the Lane-Eynon tables [9] for given conditions.

PRESENTATION AND DISCUSSION OF RESULTS

Tables 1, 2, and 3 give results of the analyses of the sorgo juices at the three stages of maturity.

Examination of the tables shows that:

(1) The sucrose, dextrose, and levulose content of the juices vary widely between varieties.

(2) The total reducing sugars and dextrose and levulose content are highest in the immature stages and decrease with maturity. In the milk stage of maturity (table 1), the total reducing sugars as invert sugar exceed the sucrose content in varieties Nos. 3, 6, 7, 8, 12, 14, 15, 16, 25, 26, and 27. The dextrose content exceeds the sucrose content in varieties 3, 6, and 27.

(3) In agreement with previous observations, the sucrose content shows large increases with maturity, and the total of reducing sugars,

TABLE 1.—*Analysis of juices of sorgo at the milk stage of maturity*

No.	Variety	Dissolved solids by Brix hydrometer	Sucrose by polarization	Apparent purity	Sucrose by—		Reducing sugars as invert sugar	Dextrose	Levulose
					Clerget method	Copper reduction			
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	S. A. 287	15.61	10.95	70.14	10.98	11.20	1.62	0.92	0.64
2	Red X	16.12	11.10	68.85	11.15	11.33	2.27	1.03	1.17
3	Indiana Amber	18.04	4.10	22.72	5.78	5.62	9.88	6.47	3.12
4	S. A. 108	14.89	6.15	41.30	7.12	7.41	4.37	2.16	1.98
5	Straight Neck	18.05	8.60	47.64	9.78	9.69	5.92	2.62	3.12
6	Gooseneck	17.19	3.95	22.97	5.23	5.32	9.72	5.49	3.94
7	S. A. 182	16.59	3.75	22.60	5.31	5.67	8.70	4.97	3.47
8	Sugar Drip	15.66	3.90	24.90	5.34	5.40	7.41	3.81	3.37
9	Planter	16.01	6.75	42.16	7.58	7.54	5.73	3.07	2.50
10	Sourless	18.75	10.95	58.40	11.69	11.33	4.44	.91	3.39
11	Mazo Amber	15.94	7.90	49.56	8.60	8.34	4.50	2.45	1.87
12	S. A. 173	15.29	3.65	23.87	5.01	5.35	7.64	4.22	3.21
13	Early Folger (16154)	18.92	11.95	63.16	11.45	11.37	4.12	4.01	0
14	S. A. 186	13.40	3.55	26.49	4.65	4.44	6.97	4.19	2.58
15	Chinese Amber	14.10	4.15	29.43	5.06	5.11	6.10	3.55	2.38
16	S. A. 119–9	13.10	4.45	33.96	4.99	5.04	5.26	3.06	2.05
17	S. A. 169	16.19	9.10	56.81	9.25	9.19	4.52	3.05	1.34
18	Jones	14.69	8.95	60.92	8.74	8.40	3.99	2.93	.94
19	White African								
20	McLean								
21	Cowper								
22	Leoti	16.66	11.75	70.52	11.82	11.53	2.24	.44	1.73
23	Early Folger (9097)	15.86	11.90	75.04	11.85	11.64	.92	.19	.70
24	Albaugh								
25	S. A. 107	16.19	3.85	23.78	5.42	5.10	8.25	3.75	4.25
26	Colman	17.24	3.05	17.69	4.89	4.94	9.07	4.59	4.22
27	Minnesota Amber	12.95	1.65	12.74	2.81	3.10	7.93	4.61	3.09
28	S. A. 183	14.26	9.30	65.21	9.19	9.24	3.17	2.35	.74
29	Honey	13.26	6.50	49.01	6.79	7.14	4.69	3.36	1.20
30	S. A. 171	16.59	9.70	58.46	9.82	9.84	4.66	3.24	1.28
31	Kansas Orange								
32	Hodo	17.10	11.40	66.66	11.24	11.32	3.78	3.61	.07
33	Iceberg								
34	Silvertop								

[9] See pp. 683–684 of reference cited in footnote 5.

dextrose, and levulose correspondingly decrease; levulose disappears in the dead-ripe stage of four varieties.

Only one variety, No. 25, shows levulose in excess of dextrose through the three stages of maturity. Two varieties, Nos. 10 and 23, show levulose in excess of dextrose in the first and second stages of maturity. Variety No. 5 shows levulose in excess of dextrose in the first and third stages. Two varieties, 2 and 22, show levulose in excess of dextrose only in the milk stage of maturity. One variety, No. 4, shows dextrose in excess of levulose in the milk stage, a reversal in the dough-to-ripe stage and another reversal in the dead-ripe stage.

With the possible exception of variety 25 in which the excess of

TABLE 2.—*Analysis of juices of sorgo at the dough-to-ripe stage of maturity*

No.	Variety	Dissolved solids by Brix hydrometer	Sucrose by polarization	Apparent purity	Sucrose by—		Reducing sugars as invert sugar	Dextrose	Levulose
					Clerget method	Copper reduction			
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	S. A. 287	17.04	12.25	71.88	11.87	12.44	1.53	1.49	0
2	Red X	15.89	11.45	72.05	11.36	11.44	1.98	1.00	.93
3	Indiana Amber	18.84	9.95	52.81	10.69	10.53	5.67	3.19	2.30
4	S. A. 108	16.11	9.60	59.59	10.14	9.97	2.46	1.06	1.33
5	Straight Neck	18.01	12.95	71.90	12.93	13.35	2.18	2.00	.12
6	Gooseneck	15.34	6.05	39.43	6.35	6.26	6.85	3.53	3.12
7	S. A. 182	18.01	11.65	64.68	11.83	12.20	3.61	2.21	1.29
8	Sugar Drip	17.61	12.90	73.25	12.98	13.32	1.95	.96	.93
9	Planter	13.71	8.70	63.45	8.49	8.68	2.78	2.07	.64
10	Sourless	17.71	13.05	73.68	13.04	12.93	1.91	.55	1.30
11	Mazo Amber	16.01	9.75	60.81	9.97	9.56	2.39	1.68	.66
12	S. A. 173	16.19	10.60	65.47	10.66	10.53	3.10	1.70	1.31
13	Early Folger (16154)	21.74	16.85	77.50	16.97	17.22	.64	.51	.11
14	S. A. 186	15.46	7.15	46.24	7.62	7.81	5.71	3.39	2.15
15	Chinese Amber	15.01	8.10	53.96	8.49	8.53	3.11	1.72	1.29
16	S. A. 119-9	14.99	8.75	58.37	8.89	9.06	3.53	2.17	1.25
17	S. A. 169	17.94	11.40	63.54	11.53	11.73	4.33	2.95	1.26
18	Jones	15.91	10.80	67.88	10.20	10.40	3.59	2.31	1.18
19	White African	16.91	12.20	72.14	11.94	11.91	1.91	1.74	.10
20	McLean	16.31	12.05	73.88	12.05	12.21	1.18	.72	.43
21	Cowper								
22	Leoti								
23	Early Folger (9097)	16.54	11.65	70.43	11.63	11.56	2.08	1.00	1.02
24	Albaugh	14.15	5.25	37.10	5.79	5.56	6.26	3.64	2.44
25	S. A. 107	16.01	7.90	49.34	8.43	8.54	4.49	1.43	1.93
26	Colman								
27	Minnesota Amber								
28	S. A. 183	14.51	8.15	56.16	8.30	8.37	3.99	2.39	1.48
29	Honey	16.41	9.95	60.63	9.52	9.74	4.76	1.75	.28
30	S. A. 171	18.31	11.90	64.99	11.71	11.83	4.76	3.42	1.20
31	Kansas Orange								
32	Hodo	17.16	11.50	67.01	11.45	11.50	3.40	1.91	1.39
33	Iceberg								
34	Silvertop								

levulose over dextrose might be a varietal characteristic, no explanation appears to be warranted from the data for the occurrence of levulose in excess of dextrose in these other instances.

Table 4 is made up from average analytical data given in tables 1, 2, and 3 for the first 18 varieties. These varieties were selected because the analytical data were complete for all 3 stages of maturity and because none of them had widely varying growing seasons likely to introduce differences due to climatic conditions during growth.

A study of the data in table 4 permits some interesting deductions as to the type of dissolved solids and the synthesis of sucrose by the sorgo plant as it matures.

TABLE 3.—*Analysis of juices of sorgo at the dead-ripe stage of maturity*

No.	Variety	Dissolved solids by Brix hydrometer	Sucrose by polarization	Apparent purity	Sucrose by—		Reducing sugars as invert sugar	Dextrose	Levulose
					Clerget method	Copper reduction			
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	S. A. 287	18.96	14.45	76.21	14.25	14.32	1.17	1.14	0
2	Red X	17.01	13.01	77.01	12.74	13.08	1.44	.95	.45
3	Indiana Amber	19.41	13.60	70.06	13.73	14.11	2.31	1.72	.52
4	S. A. 108	18.86	14.10	74.46	13.99	14.09	.93	.79	.11
5	Straight Neck	19.52	15.20	77.86	15.13	15.02	1.00	.19	.78
6	Gooseneck	14.51	6.35	43.76	5.91	6.07	6.23	4.52	1.53
7	S. A. 182	19.56	12.90	65.95	12.94	13.19	3.97	2.95	.91
8	Sugar Drip	18.86	14.65	77.67	14.67	14.30	1.03	.62	.39
9	Planter	16.50	11.85	71.81	11.77	11.47	2.32	1.60	.65
10	Sourless	19.46	15.15	77.85	15.20	15.33	1.19	.93	.23
11	Mazo Amber	17.31	11.60	67.01	11.64	11.58	1.96	1.05	.85
12	S. A. 173	19.64	13.95	71.02	14.03	14.02	2.22	1.43	.73
13	Early Folger (16154)	22.76	18.35	80.62	18.08	17.87	.56	.28	.25
14	S. A. 186	16.06	10.65	66.31	10.48	10.69	3.49	2.51	.78
15	Chinese Amber	17.54	10.75	61.28	11.41	11.60	2.72	1.60	1.04
16	S. A. 119–9	16.59	10.60	63.89	11.02	10.80	2.78	1.82	.88
17	S. A. 169	18.00	12.10	67.22	11.92	12.11	3.81	3.18	.52
18	Jones	15.30	10.35	67.64	9.87	9.66	3.40	2.54	.76
19	White African	18.26	13.90	76.12	13.62	13.83	1.39	1.35	0
20	McLean	16.86	12.80	75.81	11.83	12.11	1.27	1.23	0
21	Cowper	19.76	15.10	76.41	15.01	14.83	1.43	1.19	.20
22	Leoti	18.71	13.85	74.02	13.87	14.04	1.90	1.42	.44
23	Early Folger (9097)	18.11	13.70	75.64	13.54	13.65	.62	.54	.06
24	Albaugh	15.01	8.25	54.96	8.32	8.67	4.13	3.79	.22
25	S. A. 107	18.64	12.05	64.64	12.41	12.76	2.80	1.14	1.58
26	Colman	18.91	12.70	67.16	13.09	13.44	2.22	1.29	.87
27	Minnesota Amber	13.14	7.60	57.83	7.58	7.87	3.14	2.47	.59
28	S. A. 183	15.90	11.15	70.12	11.01	10.87	2.69	1.73	.89
29	Honey	16.26	10.35	63.65	10.29	10.29	3.81	2.50	1.19
30	S. A. 171	18.06	12.45	68.93	12.07	12.60	3.40	3.31	0
31	Kansas Orange	17.06	12.45	72.97	12.11	12.26	2.39	1.89	.44
32	Hodo								
33	Iceberg	21.06	16.50	78.34	16.38	16.00	2.35	1.33	.94
34	Silvertop	19.00	13.95	73.42	13.55	13.33	3.55	2.65	.07

From the milk to dough-to-ripe stage of maturity the total dissolved solids expressed by degrees Brix increase from 16.03 percent to 16.75 percent, or 0.72 persent, and the total sugars increase from 13.38 percent to 13.95 percent, or 0.57 percent. The increase in total sugars in this stage is 79.16 percent of the total solids increase.

From the dough-to-ripe stage to the dead-ripe stage of maturity the total dissolved solids expressed by degrees Brix increase from 16.75 percent to 18.10 percent, or 1.35 percent, and the total sugars increase from 13.95 percent to 15.07 percent, or 1.12 percent. The

TABLE 4.—*Sorgo juices of varieties 1 to 18, inclusive—averages and calculated data*

Stage of maturity	Dissolved solids in expressed juice, by Brix hydrometer	Sucrose in expressed juice by Clerget method	Reducing sugars as invert sugar	Total sugars—sucrose plus invert sugar		Total sugars calculated as invert sugar		Dextrose	Levulose
				In expressed juice	In percent of Brix solids	In expressed juice	In percent of Brix solids		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Milk stage	16.03	7.65	5.73	13.38	83.46	13.76	85.83	3.27	2.28
Dough-to-ripe stage	16.75	10.77	3.18	13.95	83.28	14.48	86.44	1.91	1.17
Dead-ripe stage	18.10	12.71	2.36	15.07	83.25	15.70	86.74	1.65	.63

increase in total sugars in this stage is 82.96 percent of the total solids increase. The increases in total solids and total sugars between the second and third stages of maturity are almost double those between the first and second stages; however, the solids acquired between the second and third stages are higher in sugar than those acquired between the first and second stages.

Table 4 shows that the total sugars as determined in the juice, sucrose plus invert sugar, increase with maturity yet their percentage of the Brix solids decrease slightly with maturity. When the equivalent total sugars are calculated as invert sugar in terms of percent Brix solids, we find the reverse is the case and the values increase with maturity.

The decrease in percentage of reducing sugars as invert sugar from the milk stage to the dough-to-ripe stage is 5.73 percent minus 3.18 percent (or 2.55 percent); this is not sufficient to account for a rise in sucrose from 7.65 percent to 10.77 percent (or 3.12 percent) because 3.12 percent sucrose would require 3.12 percent times 1.05, or 3.28 percent (instead of 2.55 percent) invert sugar for its synthesis.

Comparing the milk and dough-to-ripe stages of maturity, it is noted that the increase in total sugars as invert sugar, from 13.76 percent to 14.48 percent (or 0.72 percent), added to the decrease in reducing sugars as invert sugar, 2.55 percent, exactly equals the amount of invert sugar required for the sucrose synthesis, 3.27 percent.

When the dough-to-ripe and dead-ripe stages of maturity are similarly compared, the same relations hold. That is, the reduction in percentage of reducing sugars as invert sugar is not sufficient to account for the sucrose synthesized by an amount equal to the increase in total sugars, as invert sugar.

The average dextrose content of the juice exceeds the levulose content by nearly 1 percent in the three stages of maturity.

SUMMARY AND CONCLUSIONS

In a study of the sucrose, dextrose, and levulose content of 34 varieties of sorgo, it was found that:

(1) Sucrose content increased with maturity.

(2) In the milk stage of maturity the dextrose content exceeded the sucrose content in 3 varieties (Nos. 3, 6, and 27).

(3) In all but 7 varieties (2, 4, 5, 10, 22, 23, and 25), the dextrose content exceeded the levulose content for all stages of maturity investigated.

(4) The levulose disappeared in 4 varieties in the dead-ripe stage of maturity, in 1 variety in the dough-to-ripe stage, and in 1 variety in the milk stage.

A comparison of the average analytical data, at all 3 stages of maturity, for 18 varieties, having growing seasons which did not vary too widely, permit the following conclusions:

(1) The increase in total sugars and total solids in the plant between the second and third stages of maturity are nearly double the increase between the first and second stages. The total sugars, as actually determined, are an increasing proportion of the increasing total solids. The total sugars (sucrose plus invert sugar) as percent of Brix solids as determined decrease slightly with maturity, while

their percentage in the juice increases. If the total sugars at each stage of maturity are calculated as equivalent invert sugar, so as to permit absolute comparison, they represent increasing percentages of the total solids in the juices from the later stages of maturity.

(2) The average quantity of sucrose synthesized in the juice of the plants in maturing exceeds the quantity that could have been formed from the average decreases in the invert sugar content of the juices. This excess was equivalent to the increase in total sugars as invert sugar.

(3) The average dextrose content of the juices in all three stages of maturity exceeds the levulose content by nearly 1 percent. Dextrose in excess of levulose may be expected to contribute to dextrose crystallization in sorgo juices where the total reducing sugars exceed the sucrose. This excess of dextrose over levulose is not so much greater than that found in invert sugar made by inversion of sucrose as to suggest commercial processing of the sorgo plant for crystalline dextrose.

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